# ATTACHMENT II

AQIS AUSTRALIAN QUARANTINE AND INSPECTION SERVICE
DEPARTMENT OF PRIMARY INDUSTRIES AND ENERGY

T94/1660
3 April 1997

# ANIMAL QUARANTINE POLICY MEMORANDUM 1997/23

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Australian Veterinary Association
National Meat Association of Australia
National Farmers' Federation
Australian Registered Cattle Breeders' Association

## REVISED QUARANTINE REQUIREMENTS RELATING TO BOVINE BRUCELLOSIS AND TUBERCULOSIS

Requirements were finalised after consultation and circulated as AQPM 1996/22. However when incorporated in draft revised conditions for the importation of cattle from New Zealand AQPM 1996/43 on 13 August 1996 deficiencies became evident. Draft revised importation requirements were circulated as AQPM 1996/66 on 4 November 1996.

Concern was expressed by some respondents at the possible misinterpretation of the definition of a region - *part of the territory of a country* by the exporting country. To overcome this problem the definition from the OIE International Animal Health Code has been added as a footnote to both the brucellosis and tuberculosis requirements.

To remove ambiguity the phrase *The cattle originate from herds that were...* has been changed to *Immediately prior to export (introduction) the cattle resided in herds that were...*

No further amendments have been made to the brucellosis requirements but the following amendments have been made to the importation requirements relating to bovine tuberculosis:

- *for at least 2 years* has been added to the requirement that the cattle come from *officially free* herds. The cattle in an *officially free* herd have received at least 2 clean herd tests with a 6 month interval, the first being 6 months *following the eradication of bovine tuberculosis from the herd.* Depending on how this last phrase is interpreted this means that the herd is either equivalent to provisionally clear (PC) or confirmed free (CF1) under the BTEC Standard Definitions and Rules (SD&R). To maintain an *officially free* status a herd is required to give a clean result to an annual test. Therefore after a herd has been *officially free* for 2 years it will be at least equivalent to CF2 status according to the SD&R.

- Concern about the status of deer herds within the region have been addressed by adding *-and all cervine herds, in contact with cattle, have been* accredited free *of bovine tuberculosis for 2 years;*. The definition of accredited free as - *a cervine herd* accredited free *from bovine tuberculosis is one classified as free from bovine tuberculosis by the veterinary administration of the exporting country* has been added as a footnote.

- The regional freedom option *no case of bovine tuberculosis has occurred and all herds have remained* officially free *from bovine tuberculosis for 5 years* has been changed to *all bovine herds have remained* officially free *from bovine tuberculosis for at least 2 years and all cervine herds, in contact with cattle, have been* accredited free *from bovine tuberculosis for at least 2 years;* - this certification aligns the required period of time that the herd of origin had to be officially free with the first requirement, which also means that no cases of Tb had been detected for at least 3.5 years.

- The fifth double dot point under the second option stated that *99.8% of bovine herds have been officially free for the past 3 years and/or 99.9% of cattle have been in officially free herds for the past 6 years* has been aligned with the OIE Code by removing the */or* - this option was removed to overcome the problem created when 0.2 %, or less, of lower status herds contain a large percentage of the cattle in a region.

- To ensure that cattle introduced into the region are at least equivalent to CF2 status *originate from officially free herds,...* has been changed to *immediately prior to introduction, resided in herds which had been officially free for at least 2 years,...*

- To ensure that the test is applied in accordance with SD&R - *The tuberculin must be injected intradermally into the caudal fold which is examined 72 hours after injection when a positive test is indicated by any swelling, thickening or oedema of the injection site.* - has been added.

- To minimise the desensitising effects of the previous test the minimal time between the previous test and the movement tests has been extended from 60 days to 90 days.

- An alternative method of dealing with reactors to the after-import tuberculin test has been added. This option is only available with AQIS approval and allows for further testing, including the possible use of the comparative test, of valuable animals.

Thank you for your assistance with the development of these conditions.

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T94/542
24 March 1997

**Importation requirements relating to bovine brucellosis in cattle**

1 Immediately prior to export, the cattle resided in herds that were:
- *officially free** from bovine brucellosis, **and**
- in a country, or *part of the territory of a country***, in which bovine brucellosis is compulsorily notifiable and:
  - **either**
    no case of bovine brucellosis occurred, **and** all herds remained *officially free* from bovine brucellosis during the past five years;
    **or**
    the following requirements were met:
    - an official surveillance program is in place;
    - all reactors to tests for brucellosis are slaughtered and subjected to culture of appropriate tissues and lymph nodes for evidence of *Brucella abortus*;
    - all herds are assessed;
    - movement restrictions are placed on all infected herds;
    - compulsory eradication is practised and officially monitored;
    - less than 0.2% of herds are infected with bovine brucellosis;
    - no animal has been vaccinated against bovine brucellosis for at least the past 3 years, and
  - all animals introduced into the exporting country, or the *part of the territory of* the country:
    **either**
    immediately prior to introduction, resided in herds that qualified as *officially free* from bovine brucellosis,
    **or**
    have never been vaccinated and have been subjected to at least two approved tests with negative results at an interval of 30 days prior to entry to the herds (the tests are not valid in female animals which have calved during the 14 days prior to testing).

2 The cattle gave a negative result to a complement fixation test (CFT):
- within 30 days prior to export, and again
- within 30 days after import.

* an *officially free herd* is one which complies with OIE International Animal Health Code Article 3.2.1.1 - *Herd officially free* from bovine brucellosis (Attachment 1).

** *part of the territory of a country* is defined in the OIE International Animal Health Code as a *geographical or administrative entity possessing an authorised administrative veterinary organisation capable of taking and controlling the appropriate measures.*

T94/542
24 March 1997

**Importation requirements relating to bovine tuberculosis in cattle**

1 Immediately prior to export, the cattle resided in herds that were:
- *officially free** from bovine tuberculosis for at least 2 years, and
- in a country, or *part of the territory of a country***, in which bovine tuberculosis is compulsorily notifiable and:
  - either
    all bovine herds have remained *officially free* from bovine tuberculosis, for at least 2 years and all cervine herds, in contact with cattle, have been *accredited free**** from bovine tuberculosis for at least 2 years;
    or
    the following requirements can be met:
    - an official surveillance program is in place;
    - all bovine and cervine herds, in contact with cattle, are assessed;
    - movement restrictions are placed on all infected bovine and cervine herds;
    - compulsory eradication is practised and officially monitored;
    - 99.8% of bovine herds have been *officially free* for the past 3 years and 99.9% of cattle have been in *officially free* herds for the past 6 years;
    - all reactors and suspect cases are investigated by the Veterinary Administration which has the capacity to confirm diagnosis by microscopic-biological and/or cultural examination, and
  - cattle introduced into the country, or the *part of the territory of the country*:
    - immediately prior to introduction, resided in herds which had been *officially free* for at least 2 years, and
    - gave a negative result to an approved tuberculin test prior to entry.

2 The cattle gave a negative result to an approved single intradermal tuberculin test (using 0.1ml of Purified Protein Derivative [PPD] Tuberculin containing 3mg PPD per ml) within the 30 day period immediately prior to export and again within 90 days immediately after import. The tuberculin must be injected intradermally into the caudal fold which is examined 72 hours after injection when a positive test is indicated by any swelling, thickening or oedema of the injection site. The tests must be conducted not less than 90 days following any previous tuberculin test.

3 Reactors to the tests for bovine tuberculosis must be:
either
slaughtered and subjected to a detailed autopsy with histopathology and culture of lymph nodes.
- If *Mycobacterium bovis* is detected, no in-contact cattle may be imported, or released from quarantine in Australia;
- If *Mycobacterium bovis* is not detected, in-contact cattle must be retested a minimum of 90 days after the last test, with negative results, before they can be imported or released from quarantine in Australia.

or (for the after-import test only, with AQIS approval)
isolated from other cattle in the consignment (which remain in quarantine) and, after an interval of at least 90 days, subjected to further testing.

[*Note: This further testing could include a comparative test using bovine and avian tuberculin. This testing would be applied and interpreted under direction from AQIS. The fate of the retested and in-contact cattle would be determined by AQIS.*]

* an *officially free herd* is one which complies with OIE International Animal Health Code Article 3.2.3.1. - *Herd officially free* from bovine tuberculosis (Attachment 1).

** *part of the territory of a country* is defined in the OIE International Animal Health Code as a *geographical or administrative entity possessing an authorised administrative veterinary organisation capable of taking and controlling the appropriate measures.*

*** a cervine herd *accredited free* from bovine tuberculosis is one classified as free from bovine tuberculosis by the veterinary administration of the exporting country.

ATTACHMENT 1

**Animal Health Code Article 3.2.1.1.** **BOVINE BRUCELLOSIS**

*Herd officially free* from bovine brucellosis

To qualify as officially free from bovine brucellosis, a herd of cattle shall satisfy the following requirements;

1) be under official veterinary control;
2) contain no animal which has been vaccinated against bovine brucellosis during at least the past three years;
3) only contain animals which have not showed evidence of bovine brucellosis infection during the past six months, all suspect *cases* (such as animals which have prematurely calved) having been subjected to the necessary laboratory investigations;
4) all cattle over the age of one year (except castrated males) were subjected to serological tests with negative results performed twice at an interval of 12 months. The requirement is maintained even if the entire herd is normally tested every year or testing is conducted in accordance with other requirements established by the *Veterinary Administration* of the country concerned;
5) additions to the herd shall only come from herds officially free from bovine brucellosis. This condition may be waived for animals which have not been vaccinated, come from a herd free from Bovine brucellosis, provided negative results were shown following a buffered *Brucella* antigen test and the complement fixation test during the 30 days prior to entry into the herd. Any recently calved or calving animal should be retested after 14 days, as tests are not considered valid in female animals which have calved during the past 14 days.

**Animal Health Code Article 3.2.3.1** **BOVINE TUBERCULOSIS**

*Herd officially free* from bovine tuberculosis

To qualify as officially free from bovine tuberculosis, a herd of cattle shall satisfy the following requirements:

a) the herd is in a country of part of the territory of a country officially free from bovine tuberculosis; or
b) all cattle in the herd:
    1) show no clinical sign of bovine tuberculosis;
    2) over six weeks of age, have shown a negative result to at least two official tuberculin tests carried out at an interval of six months, the first test being at six months following the eradication of bovine tuberculosis from the herd;
    3) showed a negative result to an annual tuberculin test to ensure the continuing absence of bovine tuberculosis;
c) cattle introduced into the herd:
    1) have been certified by an Official Veterinarian as having shown a negative result to the tuberculin test during the 30 days prior to entry into the herd; and /or
    2) were kept in a herd officially free from bovine tuberculosis.

# ATTACHMENT III

CHAPTER 3.2.1.

# BOVINE BRUCELLOSIS
## (*Brucella abortus*)

**Preamble:** For diagnostic tests and vaccine standards, reference should be made to the *Manual*.

Article 3.2.1.1.

For the purposes of this *Code*:

***Country* or *part of the territory of a country free* from bovine brucellosis**

To qualify as free from bovine brucellosis, a country or part of the territory of a country shall satisfy the following requirements:

1) bovine brucellosis or any suspicion thereof is compulsorily notifiable in the country;

2) the entire cattle population of a country or part of the territory of a country is under official veterinary control and it has been ascertained that the rate of brucellosis infection does not exceed 0.2% of the cattle herds in the country or area under consideration;

3) the serological tests for bovine brucellosis are periodically conducted in each herd, with or without the ring test;

4) no animal has been vaccinated against bovine brucellosis for at least the past three years;

5) all reactors are slaughtered;

6) animals introduced into a free country or part of the territory of a country shall only come from *herds officially free* from bovine brucellosis or from *herds free* from bovine brucellosis. This condition may be waived for animals which have not been vaccinated and which, prior to entry into the herd, were isolated and were subjected to the serological tests for bovine brucellosis with negative results on two occasions, with an interval of 30 days between each test. These tests are not considered valid in female animals which have calved during the past 14 days.

   In a country where all herds of cattle have qualified as officially free from bovine brucellosis and where no reactor has been found for the past five years, the system for further control may be decided by the country concerned.

***Herd officially free* from bovine brucellosis**

To qualify as officially free from bovine brucellosis, a herd of cattle shall satisfy the following requirements:

1) be under official veterinary control;

2) contain no animal which has been vaccinated against bovine brucellosis during at least the past three years;

3) only contain animals which have not showed evidence of bovine brucellosis infection during the past six months, all suspect *cases* (such as animals which have prematurely calved) having been subjected to the necessary laboratory investigations;

4) all cattle over the age of one year (except castrated males) were subjected to serological tests with negative results performed twice at an interval of 12 months. This requirement is maintained even

if the entire herd is normally tested every year or testing is conducted in accordance with other requirements established by the *Veterinary Administration* of the country concerned;

5) additions to the herd shall only come from herds officially free from bovine brucellosis. This condition may be waived for animals which have not been vaccinated, come from a *herd free* from bovine brucellosis, provided negative results were shown following a buffered *Brucella* antigen test and the complement fixation test during the 30 days prior to entry into the herd. Any recently calved or calving animal should be retested after 14 days, as tests are not considered valid in female animals which have calved during the past 14 days.

***Herd free* from bovine brucellosis**

To qualify as free from bovine brucellosis, a herd of cattle shall satisfy the following requirements:

1) be under official veterinary control;

2) be subjected to either a vaccination or a non-vaccination regime;

3) if a live vaccine is used in female cattle, vaccination must be carried out between three and six months of age, in which case these female cattle must be identified with a permanent mark;

4) all cattle over the age of one year are controlled as provided in paragraph 4) of the definition of a herd of cattle officially free from bovine brucellosis; however, cattle under 30 months of age which have been vaccinated using a live vaccine before reaching six months of age, may be subjected to a buffered *Brucella* antigen test with a positive result, with the complement fixation test giving a negative result;

5) all cattle introduced into the herd come from a herd officially free from bovine brucellosis or from a herd free from bovine brucellosis, or from a country or part of the territory of a country free from bovine brucellosis. This condition may be waived for animals which have been isolated and which, prior to entry into the herd, were subjected to the serological tests for bovine brucellosis with negative results on two occasions, with an interval of 30 days between each test. These tests are not considered valid in female animals which have calved during the past 14 days.

Article 3.2.1.2.

*Veterinary Administrations* of *importing countries* should require:

for cattle for breeding or rearing (except castrated males)

the presentation of an *international animal health certificate* attesting that the animals:

1) showed no clinical sign of bovine brucellosis on the day of shipment;

2) were kept in a herd in which no clinical sign of bovine brucellosis was officially reported during the six months prior to shipment;

3) were kept in a *country* or *part of the territory of a country free* from bovine brucellosis, or were from a *herd officially free* from bovine brucellosis and were subjected to a serological test for bovine brucellosis with negative results during the 30 days prior to shipment; or

4) were kept in a *herd free* from bovine brucellosis and were subjected to buffered *Brucella* antigen and complement fixation tests with negative results during the 30 days prior to shipment;

if the cattle come from a herd other than those mentioned above:

5) were isolated prior to shipment and were subjected to a serological test for bovine brucellosis with negative results on two occasions, with an interval of not less than 30 days between each test, the second test being performed during the 15 days prior to shipment.

These tests are not considered valid in female animals which have calved during the past 14 days.

Article 3.2.1.3.

*Veterinary Administrations* of *importing countries* should require:

for cattle for slaughter (except castrated males)

the presentation of an *international animal health certificate* attesting that the animals:

1) showed no clinical sign of bovine brucellosis on the day of shipment;

2) are not being eliminated as part of an eradication programme against bovine brucellosis;

3) were kept in a *country* or *part of the territory of a country free* from bovine brucellosis; or

4) were kept in a *herd officially free* from bovine brucellosis; or

5) were kept in a *herd free* from bovine brucellosis; or

6) were subjected to a serological test for bovine brucellosis with negative results during the 30 days prior to shipment.

Article 3.2.1.4.

*Veterinary Administrations* of *importing countries* should require:

for *semen*

the presentation of an *international animal health certificate* attesting that:

1) when the semen is from an *AI centre*, the testing programme includes the serum-agglutination and complement fixation tests;

2) when the semen is not from an AI centre, the donor animals:

   a) were kept in a *country* or *part of the territory of a country free* from bovine brucellosis; or

   b) were kept in a *herd officially free* from bovine brucellosis, showed no clinical sign of bovine brucellosis on the day of collection and were subjected to a buffered *Brucella* antigen test with negative results during the 30 days prior to collection; or

   c) were kept in a *herd free* from bovine brucellosis, showed no clinical sign of bovine brucellosis on the day of collection and were subjected to the buffered *Brucella* antigen and complement fixation tests with negative results during the 30 days prior to collection; or

   d) showed no clinical sign of bovine brucellosis on the day of collection, were subjected to the buffered *Brucella* antigen and complement fixation tests with negative results during the 30 days prior to collection and no *Brucella* agglutinin was detected in the semen;

3) the semen was collected, processed and stored strictly in accordance with Appendices 4.2.1.1. and 4.2.1.2. as relevant.

Article 3.2.1.5.
(under study)

*Veterinary Administrations* of *importing countries* should require:

for *embryos*/ova

the presentation of an *international animal health certificate* attesting that:

1) when the embryos/ova come from a *collection unit*, the testing programme includes the buffered *Brucella* antigen and complement fixation tests;

2) when the embryos/ova do not come from a collection unit, the donor females:

   a) were kept in a *country* or *part of the territory of a country free* from bovine brucellosis; or

   b) were kept in a *herd officially free* from bovine brucellosis, showed no clinical sign of bovine brucellosis on the day of collection and were subjected to a buffered *Brucella* antigen test with negative results during the 30 days prior to collection; or

   c) were kept in a *herd free* from bovine brucellosis, showed no clinical sign of bovine brucellosis on the day of collection and were subjected to the buffered *Brucella* antigen and complement fixation tests with negative results during the 30 days prior to collection;

3) the embryos/ova were collected, processed and stored strictly in accordance with Appendices 4.2.3.1., 4.2.3.4. or 4.2.3.5. as relevant.

# ATTACHMENT IV

AUSTRALIA

# THE ERADICATION OF BOVINE BRUCELLOSIS FROM AUSTRALIAN CATTLE

***eradication*** of bovine brucellosis is a major achievement of animal disease control in Australia. It resulted from a combined commitment of the Australian cattle industry, the seven State/Territory governments and the Commonwealth Government.

The eradication program was part of the most complex animal health program ever undertaken in Australia. The epidemiology of the disease and the technology involved in identifying infected cattle are complex, and the size and scope of the program also provided a major challenge.

In the early 1970s when the brucellosis program commenced, Australia had some 18 million breeding cattle in about 180 000 herds spread across an area equivalent to that of mainland United States. The wide variation in climatic and topographical features made operations extremely difficult. In particular, the arid central and tropical north of Australia raised demands not previously met in this type of eradication program.

The initial objective was to achieve Provisional Freedom (disease prevalence of less than 0.2%) by 1984. Once this was achieved, the aim became the achievement of freedom from *Brucella abortus* infection in cattle and buffalo.

Reasons for embarking on the program included improved animal production, concern over human health, and the prospect of barriers to trade in meat and dairy products.

*The benefit to Australian society from the reduced occurrence of brucellosis and tuberculosis has been acclaimed as one of the major animal health achievements this century.*

A 10-year program of vaccinating high risk cattle herds was phased into an eradication program based on testing and slaughter. Abattoir monitoring, survey testing and milk ring testing were used to determine the presence of herds infected with brucellosis. Positive samples were traced back to their herds of origin. Strict movement controls ensured that disease was not spread from one herd to another.

Area declarations for freedom from brucellosis was achieved progressively: Tasmania in 1975; Western Australia in 1985; the Australian Capital Territory, New South Wales, Victoria and South Australia in 1988; and Queensland and the Northern Territory in 1989.

The first infected animal detected after the declaration of freedom was a pregnant heifer in a Queensland herd in September 1989. This animal and the cows in the mob of origin were immediately slaughtered. Another herd found with the disease at the end of 1990 was dealt with similarly.

In August 1989 the Office International des Epizooties recognised Australia as being free of bovine brucellosis.

This report records the achievement of eradication of bovine brucellosis and documents how it was done. The progressive achievement of brucellosis eradication is shown in Figure 2.


*Figure 1. Achievement of eradication in each Australian State and Territory.*

**Contents**

## BACKGROUND

Brucellosis probably entered Australia with early importations of dairy cattle. By the 1920s, it had become widely established in cattle herds throughout the country, with the highest prevalence in dairy cattle.

State and Territory departments of agriculture progressively introduced control programs from the 1930s, and vaccination programs when Strain 19 vaccine became available. Strain 19, an attenuated vaccine that prevents animals aborting, was used to reduce the prevalence of the disease. It was not until 1966 that plans for national eradication were first discussed. A recommendation of the Australian Agricultural Council that action be initiated on a national basis to eradicate both brucellosis and tuberculosis as soon as feasible, was subsequently adopted by the Commonwealth and State/Territory governments.

A national approach to eradication was deemed essential to avoid the economic and social disruption that a loss of export markets would have on beef and dairy producers, and the resulting costs to the Australian community. However, production losses, abortion and infertility losses were also recognised as significant, particularly in dairy herds. Reduced fertility, often not apparent, was also important in beef herds.

The official Australian coordinated plan for the eradication of brucellosis and tuberculosis commenced in 1970. At that time, progress towards brucellosis eradication varied considerably between States/Territories. Control schemes used before 1970 had resulted in the establishment of Accredited Brucellosis Free Herds. Owners maintained disease free herds to enable stock to move at any time without further testing.

When the national program commenced, Tasmania was virtually brucellosis free, and Western Australia had started a test and slaughter program. Other States/Territories were still vaccinating but were ready to enter a compulsory legislated test and slaughter phase.

The epidemiology of brucellosis was well understood in closer settled areas where control practices were well documented. However, in the rangelands of central and northern Australia, the epidemiology was known to vary with the nature of the country and the cattle management practices. Eradication practices had to be adapted to cope with the resulting problems. Fortunately, the prevalence of the disease was low in most of these areas.

These challenges required commitment by both government and the cattle industry over a 25-year period. Technical, stock management and operational procedures had to be developed specifically to provide for efficient and effective eradication.

## THE BRUCELLOSIS AND TUBERCULOSIS ERADICATION CAMPAIGN

Eradication of brucellosis was accomplished as part of the formal Brucellosis and Tuberculosis Eradication Campaign (BTEC).

This campaign was established in 1970 and continues to be a collaborative effort funded by the Australian cattle industry, the Commonwealth Government, and State and Territory governments. The Australian cattle industry contributes through transaction levies on the sale of cattle. Funding arrangements take account of the wide variation in environmental conditions across the country.

Standard definitions and rules were developed to provide minimum national standards for the campaign. These were agreed to by each State/Territory and formed the basis for eradication programs attuned to the special needs of each area. The definitions and rules have recently been revised to take account of changing circumstances. Each State and Territory uses these guidelines to formulate detailed operating procedures to implement the program.

By 1994, more than $760 million had been spent on the BTEC, with the cattle industry and government agreeing that current cost-sharing arrangements should continue until at least December 1997 to finalise the eradication of bovine tuberculosis.

**Bovine Brucellosis**

Bovine brucellosis is caused by infection with the bacterium *Brucella abortus*. It leads to abortion in mid-to-late pregnancy and a subsequent high rate of infertility.

Infection can occur in cattle of all ages but persists most commonly in sexually mature animals.

The eradication of brucellosis traditionally involves vaccination to reduce the prevalence of the disease within a herd and then a test and slaughter program to remove serological reactors.

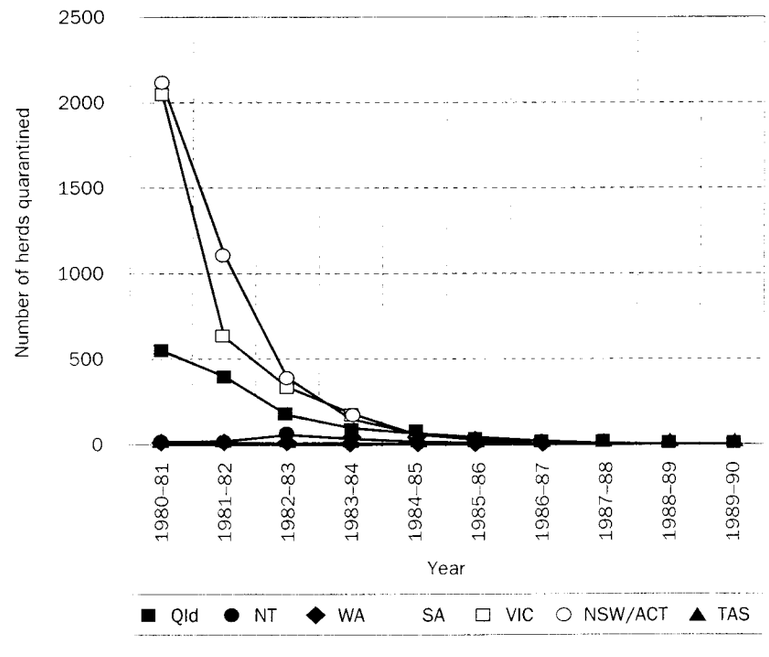


*Figure 2. Progressive achievement of brucellosis eradication in Australian States and Territories. In 1981–82 and 1982–83, the Northern Territory showed quarantine premises rather than herds.*

## Campaign Management

The management of brucellosis eradication required the cooperation of the Australian cattle industry and the State/Territory and Commonwealth governments. Each had responsibilities according to its role in disease eradication.

The Australian Constitution defines the Commonwealth Government's role in livestock disease control, and the States and Territories have the power to design and implement disease-control programs appropriate to their circumstances and legislative requirements.

To ensure full discussion of the implications of technical and resource requirements, the national Animal Health Committee (the Chief Veterinary Officers from each jurisdiction), formed a special Brucellosis and Tuberculosis Sub-Committee to handle the technical detail necessary to ensure success.

To assist in estimating the physical and financial resources required for a campaign of this size, a computer model of brucellosis in cattle herds was developed and played a key role.

Because of concern that the campaign could cause severe financial hardship for some farmers — particularly those affected by tuberculosis in remote areas — a high level BTEC Committee was established in 1985. This involved representatives of the cattle industry, specialist financial and economic advisers, and Commonwealth and State/Territory campaign managers.

Throughout the campaign, technical workshops and reviews ensured full monitoring of progress and the transfer of technical information. National progress reports documented the activities throughout Australia, and provided a basis for progressive reassessment of the procedures adopted in each State/Territory.

Operational management reviews were carried out in 1986 and 1989 to ensure the management in each jurisdiction followed the national guidelines and fully accounted for budgeted expenditure.

## Information Handling

A national network of 18 laboratory-based minicomputers was used to store and analyse data relevant to campaign management and the monitoring of progress. Called ANADIS—the Australian National Disease Information System—the system provided detailed information on the testing history of each herd, including details of status changes that followed a clear test or detection of a reactor at an abattoir. This was an important management tool for veterinarians, who used the information provided to plan future activity.

*Cattle identified with tail-tag*

## Public and Producer Awareness

Brucellosis eradication was supported from its inception by farmer organisations. Support from major stud cattle societies ensured that only cattle free of brucellosis could attend shows.

Throughout the campaign, information on the disease was widely disseminated to individual producers, to interest groups and to the general public via media outlets.

## Definitions and Rules for Conducting the Campaign

Standard definitions and rules for eradication of brucellosis played a major role in the ultimate success. These were modelled on similar guides produced by other countries and they set the standards acceptable to all States and Territories for the conduct of the campaign. They were regularly reviewed and major updates were prepared in 1986 and 1994.

The standard definitions and rules describe the requirements for both herd and area status, based on factors including the prevalence of the disease, degree of control, and vaccination requirements. These allowed progression through a series of statuses that provided increasing confidence that the disease had been eliminated.

Herd status was well understood because all States/Territories had initiated accreditation programs based on test and slaughter on an individual herd basis in their early activity against the disease.

Figure 3 shows how a herd could progress. Infected herds were tested at three-monthly intervals until clear. Further tests at least six months apart were carried out. Typically, three such tests were needed before a previously infected or suspect herd could be declared free of brucellosis. If an infected herd was found, it was quarantined and subjected to the required program of further tests.

Areas were classified according to prevalence, with each authority applying similar requirements for test procedures and quarantine control in comparable areas.

Areas started as *Control* and progressed through *Eradication* and *Provisionally Free* to *Free*. To move from one status to another, the State or Territory had to show that the area had been adequately defined, the disease prevalence was known, and an appropriate level of control or eradication activity was in place.

Movement between areas and between herds within areas was strictly supervised. Such movement was subject to testing, except where areas of equal status were involved.

## Declaration of a Free Area

The Australian Agricultural Council could declare a Free Area when:

- an approved monitoring system was in operation;
- all herds had been assessed;
- no herd was classified as Suspect, Infected, Restricted or Provisionally Clear; and
- vaccination (except with the Chief Veterinary Officer's approval) had been prohibited for at least the previous five years;
- any herds with evidence of the disease within the previous five years had tested negative at least three years after achieving Confirmed Free status or within the two years before declaration. Infected herds that achieve Confirmed Free status within two years of declaration of a Free Area needed to have tested negative at least twelve months after achieving Confirmed Free status whether before or after Free Area declaration; and
- monitoring had to be continued for five years after the declaration of a Free Area, or until no longer deemed necessary by the Animal Health Committee.

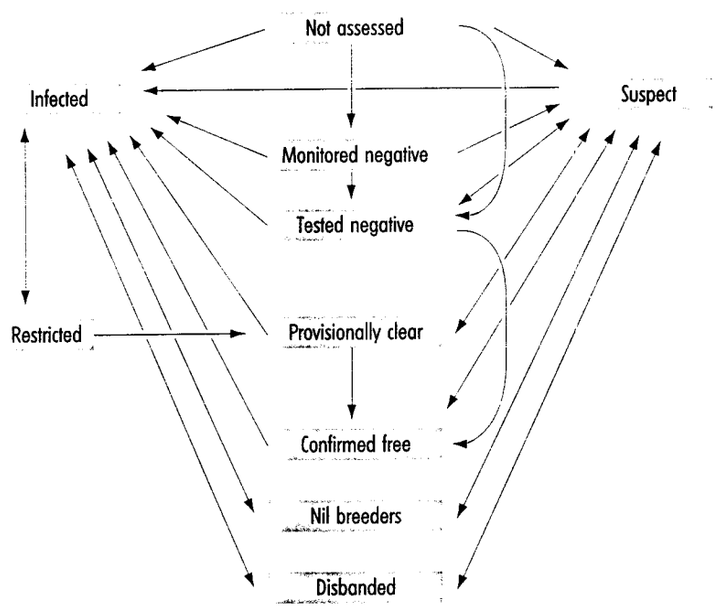


*Figure 3. Herd progression schedule.*

## Animal and Property Management

### Animal identification

A major development of the BTEC was the introduction between 1969 and 1975 of a plastic or vinyl tail-tag showing details of the animals ownership and property of origin. All cattle must be identified by the tail-tag before sale or antemortem inspection at abattoirs.

All Australian States and Territories continue to maintain a register of all properties with a cattle tail-tag number allocated to that property. Computerised records of ownership details were maintained throughout the campaign.

Animal identification and traceback of slaughter cattle were integral to successful brucellosis monitoring. The success can be assessed by figures from the latter part of the campaign. In 1989–90, more than 95% of the cattle slaughtered and inspected had a tail tag.

Audits of stock movements, such as at border crossings, saleyard inspections and abattoirs continue to ensure the proper use of tail-tags.

### Cattle movement controls

Control of stock movements is a responsibility of each State or Territory government. Before all areas achieved Brucellosis Free status, movement testing was compulsory for all stock coming from areas of lower disease status. The standard definitions and rules required all unspayed females and all breeding bulls to be tested before movement to an area of higher disease status.

In 1986, it became mandatory for herds to be kept under quarantine until Confirmed Free status was achieved.

### Herd management

In the arid and tropical rangelands of Australia, many problems were encountered in applying brucellosis eradication technology.

Management techniques, such as segregating heifers from cows at weaning, and maintaining that segregation throughout testing, were necessary. There was often no other effective control of cattle because of the vast distances, variable seasonal conditions, and lack of facilities (such as fences, yards and controlled stock waters).

## Technical Management

### Use of vaccination

Control of brucellosis was initially achieved using compulsory vaccination with strain 19 vaccine of heifer calves between the ages of 3 and 9 months. This was very efficient in reducing the abortion rate and thus reduced the spread of the disease within herds. Although the program commenced independently in each State/Territory, vaccination became widely used after 1943 following a conference of State/Territory and Commonwealth veterinarians that adopted an Australian Veterinary Association recommendation for the widespread use of Strain 19.

Strain 19 was also used for reduced-dose vaccination of adult cattle and was found to retard disease spread and allow commencement of test and

slaughter programs. In some of the more extensively grazed areas, another vaccine (strain 45/20) was used as both a preventive vaccination and in an anamnestic test that promoted a strong serological reaction.

One disadvantage of vaccination of adult cattle was that vaccination titres could interfere with laboratory tests. These difficulties were partly overcome by using more specific serological tests such as the enzyme linked immuno-sorbent assay (ELISA) and by attempting to isolate *B. abortus* from all suspect reactors.

### Abattoir monitoring

All States/Territories collected blood samples from virtually all mature breeding cattle at slaughter in abattoirs.

Government officers generally collected the blood sample, recorded the tail-tag number of the animal, and submitted the sample to the laboratory.

The full cooperation of abattoir management was essential to maintain efficient collection procedures.

An important component of tracing serological evidence of disease found in an abattoir is the correlation of carcases with the property of origin. This was generally a responsibility of abattoir management that was closely supervised by meat inspectors.

Traceback to the property of origin was a low cost strategy that was very successful.

Abattoir blood sampling for brucellosis ceased in most States/ Territories by July 1992 and in Queensland in December 1993.

### Milk ring testing

Because of its low cost and efficiency, the milk ring test (MRT) was selected for long term surveillance for brucellosis in dairy herds.

All States/Territories conducted MRTs on dairy herds at least three times a year throughout the eradication phase, and for five years after becoming brucellosis Free.

The last infected herd detected by this method was in Queensland in January 1987.

### Abortion investigations

The intensive investigation of bovine abortions was an important feature of detecting brucellosis and in monitoring progress towards eradication, particularly during the five year period when vaccination was prohibited.

### Field testing

Field testing involved hundreds of bleeding teams in all States/Territories. Blood samples were collected into tubes and sent to the nearest laboratory for testing. Mobile testing laboratories were used in several areas where it was difficult to hold cattle while samples were tested, especially in rangeland areas.

Testing cattle for brucellosis under Australian conditions required an immense effort from property managers, workers and the teams of government veterinarians and technical staff who visited properties to collect the millions of blood samples.

The logistics of identifying properties, yarding cattle, getting samples to the laboratory, maintaining accurate records, and ensuring the accurate transmission of results were complex.

### Use of laboratory tests

All States/Territories have strategically located veterinary laboratories where blood samples from both abattoir monitoring and herd sampling were tested. These laboratories were individually approved and were required to undergo rigorous quality control to maintain their participation. In 1978–79, this network of State/ Territory laboratories tested just over 11 million samples.

In the use of serological tests, Australia was fortunate in being able to build on experience gained during the successful eradication program in Tasmania.

Apart from some early use of the Serum Agglutination Test, the campaign started with the Rose Bengal Plate Test as a screening test and the Complement Fixation Test as a more specific follow-up test. This was the major test combination used for the remainder of the campaign, but individual States retained considerable discretion in the selection of tests suitable for their circumstances.

In the latter stages, an ELISA was also used. The need to distinguish between vaccination reactions and those from actual disease stimulated ongoing research into the most effective combination of laboratory tests.

The use of culture techniques to confirm the status of individual reactors was very important to determine herd status in the latter stages of the campaign. This was particularly so in herds experiencing cross-reactions from previous vaccinations or from infection with other organisms such as *Brucella suis*.

## Campaign Management

Initial funding for the campaign was partially provided by direct grants from the Commonwealth to the States/ Territories on a dollar-for-dollar basis.

Following a national inquiry in 1982 (Industries Assistance Commission) the Commonwealth entered into an agreement with each State and the Northern Territory for a comprehensive scheme to eradicate both brucellosis and tuberculosis from cattle and buffalo.

The inquiry found that although the benefits of eradication would accrue largely to the beef and dairy cattle industries, there was a case for some government assistance.

The agreed proportional funding involved a contribution for operational expenditure of 30% from each State/ Territory and 70% from the cattle industry. For compensating owners for animals destroyed, 75% was provided by the Commonwealth and 25% from the State/Territory governments. For additional assistance for cattle producers (such as a subsidy to hold cattle in yards during testing, loans for disease specific capital improvements, and funds to bring cattle back onto a depopulated property) 50% each was provided by the Commonwealth and States/Territories.

To encourage cattle producers to participate, compensation to owners for infected cattle was based on the market value of comparable but healthy animals.

Nevertheless, individual producers were responsible for costs such as

capital costs, the costs of mustering whenever herd testing was necessary, losses when slaughter compensation was not adequate, the possible cost of spelling pasture from cattle, and any costs arising from the quarantine of properties or areas.

Since the mid-1970s the cattle industry has contributed through levies on the slaughter and export of cattle.

In July 1988, the funding basis for all BTEC expenditure was rationalised to: cattle industry 50%; States 30%; and Commonwealth 20%.

A Cattle Transaction Levy was introduced in February 1991 and replaced the slaughter and export levies for cattle. The disease eradication component of this levy was set at $2.10 per head, and currently stands at $0.46 per head, as a result of decreasing campaign activity and decreasing requirements for industry funds.

Some $760 million was spent on the campaign to 1994. Industry and the State/Territory and Commonwealth governments have agreed to continue current funding arrangements for the campaign to December 1997 to complete the eradication of bovine tuberculosis.

## Achievements

All areas have now achieved brucellosis free status.

Rapid progress was made in the early days of the campaign with the number of infected herds reduced from 23% to 3% between 1976 and 1979.

The progressive improvement in brucellosis control is illustrated in Figure 4.

The level of field activity and rapid reduction in the number of brucellosis infected animals is illustrated in Figure 5.

Since completion of the campaign, ongoing brucellosis monitoring involves:

- testing imports to prevent entry of the disease;
- testing livestock exports to meet the requirements of importing countries;
- investigation of clinically suspicious events such as unusual cases of abortion; and
- a structured program of surveillance to confirm continuing freedom.

The campaign has had some additional beneficial spin-offs. Cattle property management, particularly on range-managed properties, has improved—with better control of cattle, improvements in water supply and yards, age segregation of females, new methods of identification, and better mustering techniques.

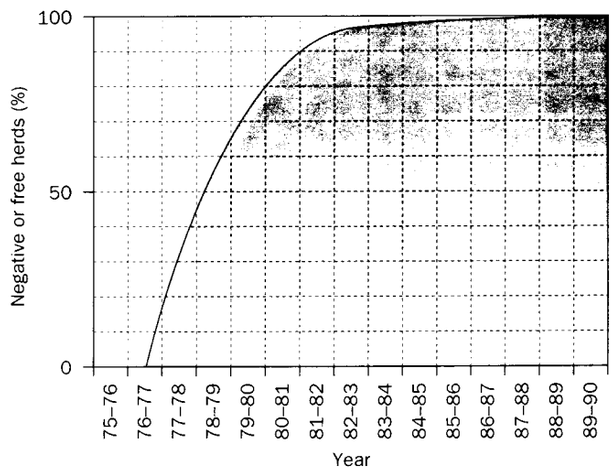


***Figure 4. The changes in percentage of free or negative herds.***

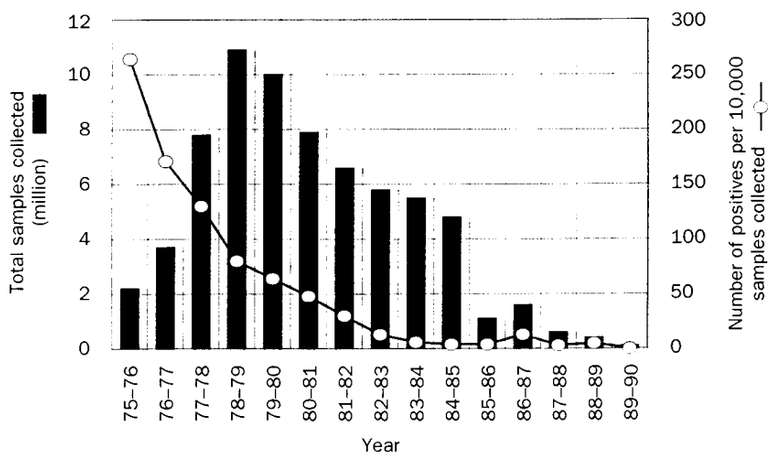


***Figure 5. Total samples collected in the field and number of positives per 10 000 samples.***

## Further Reading


Australian Bureau of Animal Health (1975) *Bovine Brucellosis and Tuberculosis National Eradication Campaign: Standard Definitions and Rules.* Australian Government Publishing Service, Canberra.

Australian Bureau of Animal Health (1976–83) *Bovine Tuberculosis and Brucellosis Eradication in Australia: Progress Reports,* nos 1–12. Australian Government Publishing Service, Canberra.

Bureau of Agricultural Economics (1987) *The Australian Brucellosis and Tuberculosis Eradication Campaign, an Economic Evaluation for Finalising the Campaign in Northern Australia.* Australian Government Publishing Service, Canberra.

Cannon, R. (1975) ANADIS: monitoring the eradication of brucellosis and tuberculosis in Australia. *Agricultural Systems and Information Technology,* 5: 11–13.

Industries Assistance Commission (1975) *Brucellosis and Tuberculosis: Bovine Brucellosis and Tuberculosis Slaughter Compensation Scheme.* Australian Government Publishing Service, Canberra.

Industries Assistance Commission (1982) *Bovine Brucellosis and Tuberculosis Eradication Campaign.* Australian Government Publishing Service, Canberra.

Tolson, J.W. and Jervois, K. (1990) *A Study to Determine the Monitoring Requirements for the Brucellosis and Tuberculosis Eradication Campaign in Australia.* Department of Agriculture, South Australia.



Produced by Arawang, Canberra, for the National Brucellosis and Tuberculosis Eradication Campaign Committee, Canberra, ACT.
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February 1996

# ATTACHMENT V

AUSTRALIAN STANDARD DIAGNOSTIC TECHNIQUES FOR ANIMAL DISEASES

STANDING COMMITTEE ON AGRICULTURE AND RESOURCE MANAGEMENT

ANIMAL HEALTH COMMITTEE

SUB-COMMITTEE ON ANIMAL HEALTH LABORATORY STANDARDS

# Bovine Brucellosis

## *Bacteriology*

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## Contents

Bovine Brucellosis

First published as: *Bovine Brucellosis Standard Bacteriological Techniques* by L. A. Corner and G. G. Alton, by the Australian Bureau of Animal Health (1982).

Reprinted by CSIRO for the Australian Agricultural Council (1987) as: Australian Standard Diagnostic Techniques for Animal Diseases, No. 4, *Bovine Brucellosis – Standard Bacteriological Techniques.*

## 1. Introduction

In the past cultural examination for the diagnosis of bovine brucellosis was considered unreliable. The bacteria (*Brucella abortus*) are often present in the tissues only in small numbers and it has been difficult to obtain uncontaminated specimens. The development of highly selective media and the means for easy maceration of tissue have improved the prospects for successful cultures.

Bacteriological diagnosis can be used to determine the infection status of animals in problem herds and to validate serological procedures. It is axiomatic that isolation of *Brucella abortus* is the only criterion of a definitive diagnosis.

## 2. Culture of Brucella

Solid media are preferred for the culture of brucella as they facilitate recognition and discourage dissociation but liquid media permit the culture of larger volumes than can be conveniently dealt with on solid media.

### *2.1. Basal Media*

A range of commercial dehydrated brucella media is available (see 6.1.). The medium we prefer is Trypticase soy (TS)(BBL Division of Becton, Dickinson & Co., distributed in Australia by Becton Dickinson, see 6.1.) broth to which is added 1.5% agar for solid medium. Bovine serum (5%) is required for the isolation and growth of *B. abortus* biotype 2 and is, therefore, added routinely. We use bovine serum but equine serum may be used. The serum should be free from brucella antibodies, and is inactivated at 56°C for 30 min and passed through a 0.22 µm sterilising filter. The medium is autoclaved, and cooled to 56°C before the serum is added.

Serum dextrose (SD) agar plates are used when specimens likely to contain *B. abortus* strain 19 are being examined. For this, nutrient agar is prepared, autoclaved, cooled to 56°C and 5 mL of a filter-sterilised stock solution of serum containing 20% dextrose, added per 95 mL of media.

### *2.2. Selective Media*

The selective media may be stored for up to four weeks at 4°C in sealed containers. Preparation of stock solutions of the antibiotics is given in 6.2.

#### 2.2.1. Solid Medium

In the preparation of selective agar medium (Kuzdas and Morse, 1953) the following substances are added per litre of basal medium: 100 mg of cycloheximide; 25 000 units of bacitracin and 6000 units of polymyxin B sulfate. Plates should contain at least 25 mL of medium.

#### 2.2.2. Liquid medium

The antibiotics for the selective broth medium are similar to those of Brodie and Sinton (1975) and are given below, in quantities per litre of medium.

| | |
|---|---|
| Cycloheximide | 100 mg |
| Bacitracin | 25 000 units |
| Polymyxin B sulfate | 6000 units |
| Nalidixic acid | 5 mg |
| Vancomycin | 20 mg |
| Amphotericin B | 1 mg |
| D-cycloserine | 100 mg |

At the concentrations listed above there is no inhibition of any *B. abortus* biotypes.

#### 2.2.3. Biphasic medium

A medium consisting of both a solid and a liquid phase in the same bottle, similar to that described by Castaneda (1947) for blood cultures, is recommended for culture of materials such as milk and macerated tissues.

Sterile plastic tissue culture flasks (50 mL) are used [Disposable Products, 16 Park Way, Technology Park, SA 5099. Tel. (08) 349 6555; (008) 803 308. Fax (008) 806 073]. After the TS agar medium has been sterilised, cooled and the seven antimicrobial agents listed above and serum added, 8 mL are dispensed into each flask. The flask is laid flat on a narrow side while the medium solidifies. Freshly prepared selective broth is dispensed to the flasks in 9 mL amounts. Detachment of the agar from the side of the flask will not occur if the flask is held at room temperature (20–25°C) between setting of the agar and addition of the broth. These operations should be done no more than 24 hours apart.

### *2.3. Testing of Culture Media*

It is essential to test the ability of each batch of culture medium to support the growth of *B. abortus*. This is best done by inoculating a few plates of each batch with a known number of cells (about 100) of the fastidious, serum-requiring *B. abortus* biotype 2. If the medium will support the growth of biotype 2 it will support all the other biotypes. The biphasic medium is inoculated with a similar number of cells of the same organism. For this a stock of freeze-dried culture containing a convenient number of brucella organisms per ampoule may be prepared. If this is not possible, appropriate dilutions of a 24 hour brucella culture can be made in peptone-saline and, if stored at 4°C these will give reproducible results up to one week after preparation. Whenever possible, testing of a new batch of medium should be done in parallel with a batch of known quality.

### *2.4. Incubation*

All cultures are incubated at 37°C in air containing 5–10% carbon dioxide except for the SD agar plates used to isolate strain 19, which are incubated in air. Plates are incubated with the agar uppermost and flasks with the agar slope vertical. Flasks are incubated with the caps loose. Plates are examined after four and eight days. The flasks containing biphasic medium are

examined every four to seven days for up to 28 days. After examination, the flasks are tilted so that the liquid phase runs over the solid phase, then righted and returned to the incubator.

### *2.5. Identification of Isolates*

Brucella colonies after four to five days incubation are round with smooth margins and are 2–4 mm in diameter. Viewed through transparent medium the colonies are translucent and a pale honey colour. Viewed from above the colonies are pearly white and convex. Brucella are Gram-negative coccobacilli and are stained red by the modified Ziehl–Neelsen method.

Tentative identification of isolates may be made using the slide agglutination test. A quantity of growth from an agar slope or portion of a large colony is dispersed in a drop of saline. To this a drop of diluted antiserum to smooth brucella is added and the mixture examined for agglutination. A control test using normal serum should be run to detect non-specific agglutination.

Isolates considered to be brucella on the basis of colonial morphology and slide agglutination tests can be submitted for typing. Send specimens to the National Brucellosis Reference Centre, Australian Animal Health Laboratory, PO Box 24, Geelong, Vic. 3220. Tel. (052) 275 000; Fax (052) 275 555.

## 3. Collection of Specimens for Cultural Examination

Success in culturing specimens depends on careful preparation and handling during transport. Specimens are collected using aseptic techniques and placed in sterile leak-proof containers of appropriate size to limit the amount of contained air which occupies unnecessary space and impedes refrigeration. For these reasons sterile plastic bags such as Whirl-paks (available from Nasco Industries Inc., Fort Atkinson, Wis., USA, distributed in Australia by Leader Products Pty Ltd, Craigieburn, Vic. 3064) are ideal for this purpose. Specimens should be refrigerated immediately they are collected and frozen if they cannot be cultured within 24 hours. For transport, the specimens are packed in stout polystyrene foam containers. If the specimens are already frozen they should be transported packed on dry ice to ensure that they remain frozen.

When sealing the Whirl-pak plastic bags, first squeeze out most of the air, then roll up the bag tightly around the wire and bend the wires over to maintain the seal.

### *3.1. Milk*

Since infection of the udder may be confined to one quarter, 10–20 mL of milk is obtained from each quarter separately. The whole udder is washed and dried, and the tip of each teat is disinfected with a swab of 70% ethanol ($C_2H_5OH$). The first one or two streams of milk are discarded and then a sample from each teat is milked directly into a sterile container. Sterile plastic or glass jars or Whirl-pak plastic bags are suitable containers for milk. It is essential to avoid contact between the stream of milk and the milker's hand, both to protect the milker and to prevent cross contamination of specimens.

Resting mammary fluid is also valuable for bacteriological examination. After disinfecting the udder, all the fluid expressed from each teat is collected as there may be only a very small volume of secretion present.

### *3.2. Vaginal Swabs*

Brucella may be recovered from a vaginal swab taken in the six-week period following parturition or abortion. A guarded sterile swab, e.g. sterile guarded mare swab (available from Cenvet, Crows Nest, NSW 2065, Australia) is used and the swab should then be broken off in a sterile container if it is to be cultured immediately or into a vial of transport medium (see 6.3.) if there is to be a delay before the swab is cultured.

### *3.3. Animal Carcases*

The tissues from which brucella can most often be isolated are those of the reticuloendothelial system, plus either the contents of the pregnant or post-parturient uterus, the udder and its secretions, or the male reproductive tract (see 6.4. and 6.5.).

The equipment required for collecting the specimens, besides the usual protective clothing, gloves, knives etc., are:

(a) A steriliser and two perforated inner trays;
(b) 12 pairs of surgical scissors (15 cm), 12 pairs of rat-toothed forceps (15 cm) and two No. 4 scalpel handles and large blades;
(c) sterile pasteur pipettes or a 20 mL syringe and 18 gauge needles;
(d) a means of heating the steriliser such as a portable gas barbeque or stove-type gas burner;
(e) a gas torch for flaming the tissue;
(f) wet or dry ice, or an ample supply of 'cold bricks'; and
(g) sterile specimen containers.

After the animal is slaughtered and prior to removal of the skin the udder is removed, so that the mammary lymph nodes are removed with it. When removing the head, cut through the trachea 5–8 cm caudal to the larynx. This will ensure that the mandibular (syn. submaxillary) and medial retropharyngeal lymph nodes are left intact on the head.

Specimens for bacteriological examination are collected aseptically. For each tissue a separate set of sterile forceps and scissors is used. The tissue overlying and surrounding the lymph nodes or tissue to be collected is flamed prior to

removal of the specimen. After removal the specimen is placed in a sterile container.

When collecting lymph nodes they should be dissected free from the surrounding fat but every endeavor must be made to avoid cutting the capsule of the lymph node. Samples from the right and left sides are kept separate. A suitable specimen from the spleen would be about 10 g.

The tissues for cultural examination are listed in 6.4. and 6.5. The tissues are also listed in order of frequency of infection in infected animals (6.6. and 6.7.) (Corner *et al.*, 1987).

### *3.4. Foetus*

From an aborted foetus or one taken from the uterus of a slaughtered reactor, samples of spleen, lung (both left and right) and stomach contents, 10–20 mL, are collected (see 6.4.). During collection the same aseptic precautions as described above are used. A sample of stomach contents may be withdrawn with a pasteur pipette or sterile syringe.

### *3.5. Foetal Membranes*

One or two cotyledons are collected from the foetal membranes of a pregnant uterus of a slaughtered reactor or from the aborted membranes. In abortion due to brucella infection, parts of the foetal membranes may contain large numbers of brucella organisms; this is also true of membranes from infected full-term births. Brucella may also be isolated in large numbers from healthy looking cotyledons. Some infected cotyledons lose their normal bright red appearance and become a dirty greyish–yellow colour. The membranes should be examined carefully and the least healthy-looking cotyledons, or portions of them, should be removed and transferred to the laboratory for culture.

## 4. Culture of Specimens

Brucella are often present in very small numbers in tissues and milk, thus the chances of isolating the organism are improved by increasing the number of plates inoculated per sample or, more conveniently, by using a selective biphasic medium in addition to one or more plates of solid medium. For each specimen we inoculate one plate of solid selective medium, and two flasks of biphasic medium. The plate medium is included as it may give an earlier positive result than the biphasic medium. Macerated tissue or fluid specimen is inoculated onto the plate of solid medium using a sterile swabstick. The two flasks of biphasic medium are inoculated with a pasteur pipette; the first inoculated with 1 mL of inoculum and the second with 2 mL.

Calculations made from the results of culturing 8600 specimens from 440 reactor cattle by the above method, indicated that there was a 95% chance that an infected specimen would be detected. In addition, subsets of specimens which maximise the detection rate for a given amount of effort and using the same culture method were established for cows (see 6.8.) and heifers (see 6.9.). The detection rate might be increased either by increasing the volume of inoculum for specimens, or by culturing extra tissues in addition to those in the recommended subset. Because of the high sensitivity of the method, it seems better to include additional tissues than to use larger inocula from those in the subset.

For samples from cattle vaccinated with *B. abortus* strain 19 a plate of selective SD agar should be inoculated and incubated in air. Strain 19 grows better on SD agar plates than on TS agar plates but grows as well as virulent biotypes in the biphasic medium. A combination of SD agar plates and TS agar plates allows the identification of mixed infections of Strain 19 and virulent *B. abortus*.

For the initial culture, only half the sample is used, the other half being frozen for later examination in case the initial cultures become overgrown by contaminants. Where the specimen submitted is tissue, intact tissue should be stored frozen as the viability of brucella in frozen macerated tissue suspensions is poor. The number of viable brucella in tissues stored frozen at -20°C remains constant for at least 18 months. If unprocessed tissues that have been frozen and thawed are refrozen, then thawed a second time, there is a small loss of viability, but if the process is repeated the viability of the bacteria falls sharply (L.A. Corner and G.G. Alton, unpublished data 1982).

Frozen specimens are thawed at room temperature or overnight at 4°C.

### *4.1. Milk*

The milk samples from each quarter are cultured separately to avoid failure to detect infection by the effects of dilution. Centrifuge 10–20 mL for 20 min at 6000–7000 *g*. After centrifugation collect a portion of the cream layer on a sterile cottonwool swabstick. Discard the skim milk and remaining cream and retain the sediment. Inoculate an agar plate by smearing a mixture of the cream and the sediment over the whole surface of the plate with the swabstick. After inoculating the plates, break off the swab into a flask of biphasic medium. Alternatively 1 or 2 mL of whole milk may be added to a flask of biphasic medium.

Gravity cream may be cultured. Milk samples in sterile test tubes or sterile universal bottles are held at 4°C overnight and the cream is cultured as described above. This method gives similar results to that described above but there is a risk that contaminants may proliferate in the sample during storage.

### 4.2. Other Liquids

Foetal stomach contents and synovial fluid are spread directly on solid selective medium with a swabstick and pipetted into biphasic medium. Culture of blood may also be attempted (see 6.10.).

### 4.3. Tissue

Tissues with gross surface contamination should be flamed or dipped in ethanol before flaming. Using sterile instruments the superficial fat is trimmed from the specimen. Half the tissue specimen or up to 10 g of the tissue is sliced into small pieces. The pieces are placed in a sterile plastic bag and macerated in 2–10 volumes of sterile isotonic phosphate buffered saline (PBS) pH 6.3 (see 6.12.) or nutrient broth, in a Colworth stomacher (FSE Pty Ltd). This operation is best conducted in a Class 2 biohazard cabinet. Other methods for the preparation of tissue for culture are listed in 6.11.

### 4.4. Foetal Membranes

Aborted foetal cotyledons frequently show gross contamination with soil or bedding and should therefore be washed before culture. They are rinsed in at least three changes of sterile saline or PBS pH 6.3 before being macerated. To decrease the risk of contaminants overgrowing the plate, 10-and 100-fold dilutions of the macerated tissues are cultured. The dilutions are made in PBS pH 6.3. Alternatively by using a swabstick, a small amount of inoculum from one plate can be applied to the second plate. Similarly a third plate can be inoculated from the second.

### 4.5. Vaginal Swabs

If the swab is submitted in a sterile tube, the end of the tube from which the swab is to be withdrawn is flamed, the swab removed and rubbed over the surface of the medium. The swab may then be placed in a flask of biphasic medium.

If the swab is submitted in transport medium, a plate is inoculated with the swab. The swab is then placed in a flask of biphasic medium.

### 4.6. Isolation of Brucella by Laboratory Animal Inoculation

Laboratory animal inoculation for the primary isolation of *B. abortus* is not recommended. Culture methods outlined above are at least as sensitive as animal inoculation.

## 5. References


Brodie, J., and Sinton, G.P. (1975). Fluid and solid media for isolation of *Brucella abortus*. *Journal of Hygiene* 74(3), 359–67.

Castaneda, M.R. (1947). A practical method for routine blood culture in brucellosis. *Proceedings of the Society for Experimental Biology and Medicine* 64, 114–15.

Corner, L.A., Alton, G.G., and Iyer, H. (1987). Distribution of *Brucella abortus* in infected cattle. *Australian Veterinary Journal* 64, 241–4.

Kuzdas, C.D., and Morse, E.V. (1953). A selective medium for the isolation of Brucellae from contaminated material. *Journal of Bacteriology* 66, 502–4.

Plant, J.W., Claxton, P.D., Jakovljevic, D., and De Saram, W. (1976). *Brucella abortus* infection in the bull. *Australian Veterinary Journal* 52, 17–20.


## 6. Appendixes

### 6.1. Appendix 1 — Commercially Available Brucella Media

#### 6.1.1. Trypticase–soy

*BBL Division of Becton, Dickinson & Co.* Cockeysville, MD USA. Agent: Becton Dickinson, 80 Rushdale Rd, Knoxfield, Vic. 3180. Tel. (03) 764 2444; Fax (03) 764 2550.

#### 6.1.2. Bacto–tryptone

*N.B.* 'Peptone 140' is an equivalent product according to the manufacturer.

*Difco Laboratories*. Detroit, Michigan, USA. Agents: Helena Laboratories (Aust.) Pty Ltd, Mt Waverley, Vic. 3150. Tel. (03) 543 7299, (008) 033 137; Fax (03) 543 7542. FSE Pty Ltd, 47–49 Overseas Drive, Noble Park, Vic. 3174. Tel. (03) 795 0077; Fax (03) 790 1900.

#### 6.1.3. Tryptic Soy Agar and Broth/Brucella Agar and Broth

*Gibco Diagnostics Laboratories*. Madison, Wisconsin, USA. Agents: Helena Laboratories (Aust.) Pty Ltd (see 6.1.2.).

#### 6.1.4. Tryptone soya/Brucella medium

*Oxoid (Australia)*. Heidelberg West, Vic. 3081. Tel. (03) 458 1311, (008) 331 163; Fax (03) 458 4759.

We prepare nutrient agar from Bacto peptone (Difco) and beef extract (Lab Lemco Powder, Oxoid). Dehydrated nutrient agars often called blood agar base or Columbia agar, are available from numerous commercial sources.

### 6.2. Appendix 2 — Preparation of Antibiotic Stock Solutions

Convenient stock solutions of antibiotics may be prepared as follows.

#### 6.2.1. Cycloheximide

*Sigma-Aldrich*. Unit 2, Anella Avenue, Castle Hill, NSW 2154. Tel. (02) 899 9977; Fax (008) 800 096. Cycloheximide powder (1 g) is dissolved in 100 mL of distilled water and passed through a 0.22 µm Millipore filter. Add 10 mL of stock solution per litre of melted and cooled medium. Alternatively, the powder can be dissolved in 5 mL of acetone ($CH_3COCH_3$) and then diluted in distilled water to make 100 mL. No sterilisation is required. The solution is relatively stable and may be stored in a domestic refrigerator for up to six months.

#### 6.2.2. Bacitracin

*Sigma-Aldrich* (see 6.2.1.); other brands. This antibiotic is supplied in bottles of sterile powder, or as tablets each containing 2500 units/mL of bacitracin, 10 mL is added per litre of medium. If the entire amount is not immediately required it may be divided into aliquots and stored, frozen, for up to two weeks.

#### 6.2.3. Polymyxin B sulfate

*Wellcome Pharmaceuticals, UK.* Agents: Murex Diagnostics, 53 Phillips Street, Cabarita, NSW 2137. Tel. (008) 819 289; Fax (02) 743 6759. Calbiochem-Novabiochem Pty Ltd, PO Box 140, Alexandria, NSW 2015. Tel. (02) 318 0322, (008) 023956; Fax (02) 319 2440.

Dissolve 500 000 units (the contents of 1 vial) in 50 mL of water and add 0.6 mL/L of medium. Surplus stock solution may be kept frozen for up to one month; it should not be refrozen after thawing.

#### 6.2.4. Vancomycin

*Sigma-Aldrich*, see 6.2.1. Dissolve in sterile water to give 50 mg/mL and add 0.4 mL of stock solution per litre of medium. Surplus stock solutions should be discarded.

#### 6.2.5. Nalidixic Acid

*Sigma-Aldrich*, see 6.2.1. Dissolve in 0.5 mol/L sodium hydroxide solution at the rate of 5 mL/mL, then add 1 mL stock solution per litre of medium.

#### 6.2.6. Amphotericin B

*Fungizone, Squibb*; Bristol-Myer-Squibb, Noble Park, Vic. 3174. Tel. (03) 213 4231; Fax (03) 701 1334.

Dissolve in sterile water at 1 mg/mL and add 1 mL of stock solution per litre of medium. Surplus stock solution can be stored in the dark at 4°C for up to six months.

#### 6.2.7. Cycloserine

*Sigma-Aldrich* (see 6.2.1.); other brands. Dissolve in sterile water at 50 mg/mL and add 2 mL of stock solution per litre of medium. Surplus stock solutions should be discarded.

#### 6.2.8. Commercial Antibiotic Mixture

A freeze-dried antibiotic supplement for the isolation of brucella species is available commercially (Oxoid Brucella Supplement SR83).

### *6.3. Appendix 3 — Brucella Transport Medium (D. Pietz, pers. comm. 1982)*

Trypticase soy or Tryptone broth (0.5 mL) in a 100 x 13 mm capped tube.

After collection the tip of the swab is inserted into the tube and the shank of the swab cut off and the cap replaced. The tube is transported to the laboratory under refrigeration and a plate of medium inoculated with the swab which is then placed in a flask of biphasic selective medium.

### *6.4. Appendix 4 — Specimens Collected from Cows and Heifers*

#### 6.4.1. Head

(a) Parotid lymph node (l.n.) (left and right).
(b) Mandibular (Submaxillary) l.n. (left and right).
(c) Medial retropharyngeal l.n. (left and right).

#### 6.4.2. Body

(a) Caudal superficial cervical (prescapular, suprascapular) l.n. (left and right).
(b) Subiliac (prefemoral, precrural) l.n. (left and right).
(c) Medial iliac (internal iliac) l.n. (left and right).
(d) Mammary (supramammary, superficial inguinal) l.n. (left and right).

#### 6.4.3. Visceral Organs

(a) Caudal (posterior) mediastinal l.n.
(b) Mesenteric l.n. (duodenal, jejunal and ileal regions).
(c) Hepatic l.n.
(d) Spleen (5–10 g).
(e) Uterus (caruncles) or cotyledon.
(f) Udder (if lactating or has been lactating. Samples from all quarters.)
(g) Milk or resting mammary fluid (as for udder).

#### 6.4.4. Foetal Tissues

(a) Spleen.
(b) Lung (left and right).
(c) Stomach contents (10–20 mL).

The following additional specimens may be collected for culture from particular animals: vaginal swab, blood and synovial fluid.

### *6.5. Appendix 5 — Specimens collected from Bulls (L.A. Corner, G.G. Alton and H. Iyer, unpublished data 1986)*

#### 6.5.1. Head

(a) Parotid l.n. (left and right).
(b) Mandibular l.n. (left and right).
(c) Medial retropharyngeal l.n. (left and right).

#### 6.5.2. Body

(a) Caudal superficial cervical l.n. (left and right).
(b) Subiliac l.n. (left and right).
(c) Medial iliac l.n. (left and right).
(d) Scrotal (superficial inguinal) l.n. (left and right).

#### 6.5.3. Visceral Organs

(a) Caudal mediastinal l.n.
(b) Mesenteric l.n. (duodenal, jejunal and ileal regions).
(c) Hepatic l.n.
(d) Spleen (5–10 g).
(f) Testes (left and right).
(g) Epididymis (left and right).
(h) Ampulla (left and right).
(i) Seminal vesicle (left and right)

6.5.4. Decreasing Order of Importance of Specimens from Bulls (Plant *et al.*, 1976)

(a) Ampulla.
(b) Mandibular l.n.
(c) Medial iliac l.n.
(d) Subiliac l.n.
(e) Caudal superficial cervical l.n.
(f) Medial retropharyngeal l.n.
(g) Seminal vesicle.
(h) Testis.
(i) Epididymis.
(j) Paratid l.n.
(k) Caudal mediastinal l.n.
(l) Spleen.

The following additional specimens may be collected for culture from particular animals: semen, blood and synovial fluid.

*6.6. Appendix 6 — Specimens yielding* Brucella abortus *from known infected cows (from Corner et al. 1987)*

See Table 1.

*6.7. Appendix 7 — Specimens yielding Brucella abortus from known infected heifers (from Corner et al., 1987)*

See Table 2.

*6.8. Appendix 8 — Subset of tissues for cows that maximise detection of infection for a given amount of effort (from Corner et al., 1987)*

See Table 3.

*6.9. Appendix 9 — Subset of tissues for heifers that maximise detection of infection for given amount of effort (from Corner* et al. *1987)*

See Table 4.

**Table 1.** Specimens yielding *Brucella abortus* from known infected cows (from Corner *et al.* 1987)

| *Specimen* | *No. examined* | *No. positive on culture (%)* |
|---|---|---|
| Lymph nodes | | |
| Parotid | 123 | 60 (49) |
| Mandibular (Submaxillary) | 122 | 61 (50) |
| Medial retropharyngeal | 123 | 83 (67) |
| Caudal superficial cervical (prescapular) | 123 | 65 (53) |
| Caudal mediastinal | 122 | 37 (30) |
| Hepatic | 87 | 15 (18) |
| Jejunal mesenteric | 85 | 6 (7) |
| Medial iliac | 123 | 89 (72) |
| Subiliac (prefemoral) | 123 | 111 (90) |
| Mammary (Superficial inguinal) | 123 | 111 (90) |
| Spleen | 122 | 24 (20) |
| Udder | 88 | 74 (84) |
| Uterine caruncle | 101 | 47 (47) |
| Milk | 102 | 86 (84) |
| Foetal tissue | 19 | 5 (26) |
| Foetal membranes (cotyledons) | 19 | 3 (16) |

**Table 2.** Specimens yielding *Brucella abortus* from known infected heifers (from Corner *et al.*,1987)

| *Specimen* | *No. Examined* | *No. Positive on Culture (%)* |
|---|---|---|
| Lymph nodes | | |
| Parotid | 61 | 39 (64) |
| Mandibular (submaxillary) | 61 | 43 (71) |
| Medial retropharyngeal | 61 | 38 (62) |
| Caudal superficial cervical (prescapular) | 61 | 38 (62) |
| Caudal mediastinal | 61 | 30 (49) |
| Jejunal mesenteric | 61 | 8 (33) |
| Medial iliac | 61 | 35 (57) |
| Subiliac (prefemoral) | 61 | 38 (62) |
| Mammary (superficial inguinal) | 61 | 34 (56) |
| Spleen | 61 | 30 (49) |
| Liver | 23 | 4 (17) |
| Lung | 22 | 5 (23) |
| Kidney | 22 | 5 (23) |
| Uterine caruncle | 44 | 9 (21) |
| Udder | 22 | 5 (23) |
| Milk | 6 | 6 (100) |
| Foetal membranes | 13 | 2 (15) |
| Foetal tissue | 13 | 2 (15) |

*N.B.* A heifer is defined here as an animal <18 months of age i.e. having no permanent incision teeth.

**Table 3.** Subset of tissues for cows that maximise detection of infection for a given amount of effort (from Corner *et al.*, 1987)

| *Tissue* | *Cumulative No. detected (total 137)* |
|---|---|
| Mammary l.n[A] | 121 |
| Mandibular l.n. | 129 |
| Superficial cervical l.n. | 132 |
| Medial iliac l.n. | 134 |
| Parotid l.n. | 135 |
| Subiliac l.n. | 136 |
| Uterine caruncle or foetus or cotyledon | 137 |

[A] l.n. = lymph node.

**Table 4.** Subset of tissues for heifers that maximise detection of infection for given amount of effort (from Corner *et al.* 1987)

| *Tissue* | *Cumulative No. detected (total 137)* |
|---|---|
| Mandibular l.n.[A] | 45 |
| Parotid l.n. | 49 |
| Medial retropharyngeal l.n. | 52 |
| Uterine caruncle or foetus or cotyledon | 55 |
| Caudal superficial cervical l.n. | 57 |
| Mesenteric l.n. | 58 |
| Spleen | 59 |
| Medial iliac l.n. | 60 |
| Subiliac l.n. | 61 |

[A] l.n. = lymph node.

*6.10. Appendix 10 — Blood Culture*

Blood may be cultured on solid medium, with or without preliminary treatment by freezing and thawing. However, the culture of blood is best done in liquid medium. The blood sample, about 10 mL, contained in the syringe or vacuum collecting tube is transferred aseptically to a biphasic medium flask. If blood is not mixed

with anticoagulant when drawn it must be transferred immediately to the biphasic medium flask and gently mixed with the broth containing 2% sodium citrate $C_6H_5Na_3O_7$).

After inoculation, the flasks are incubated in an upright position and examined every four to seven days for at least 28 days.

### *6.11. Appendix 11 — Alternative Methods of Preparing Tissue for Culture*

If a Colworth stomacher [A.J. Seward, distributed by Townsend and Mercer Pty Ltd (branches in all States)] is unavailable the following procedures may be adopted. These are listed in decreasing order of merit (for cultural effectiveness).

(a) A tissue grinder (Griffith tissue grinder).
(b) A tissue blender (rotating blade type).
(c) Pestle and mortar, with or without the addition of sterile sand.
(d) Flame the sample; cut through the sample and rub the exposed surface on the agar plate.

### *6.12. Appendix 12 — Phosphate Buffered Saline pH 6.3*

Phosphate buffered saline of pH 6.3 is prepared as follows. Dissolve 0.5 g sodium dihydrogen phosphate dihydrate ($NaH_2PO_4.2H_2O$), 0.22 g of anhydrous disodium hydrogen phosphate ($Na_2HPO_4$) and 8.5 g of sodium chloride in 1 L of distilled water.

AUSTRALIAN STANDARD DIAGNOSTIC TECHNIQUES FOR ANIMAL DISEASES

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# Bovine Brucellosis

## *Serology*

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## Contents

The standard diagnostic techniques for the serological diagnosis of bovine brucellosis were previously published under five separate titles.

*Complement Fixation Test for Bovine Brucellosis*, by Working Party of Laboratory Officers, first published by the Australian Bureau of Animal Health (1975).

Reprinted by CSIRO for the Australian Agricultural Council (1987) as: Australian Standard Diagnostic Techniques for Animal Diseases, No. 2, *CFT – Bovine Brucellosis*.

*Milk Ring Test for Bovine Brucellosis*, by Working Party of Laboratory Officers, first published by the Australian Bureau of Animal Health (1975, revised in 1978).

Reprinted by CSIRO for the Australian Agricultural Council (1987) as: Australian Standard Diagnostic Techniques for Animal Diseases, No. 1, *Milk Ring Test – Bovine Brucellosis*.

*Rose Bengal Test for Bovine Brucellosis*, by Working Party of Laboratory Officers, first published by the Australian Bureau of Animal Health (1975).

Reprinted by CSIRO for the Australian Agricultural Council (1987) as: Australian Standard Diagnostic Techniques for Animal Diseases, No. 5, *Rose Bengal Test – Bovine Brucellosis*.

*Brucellosis Serum Agglutination Test*, by Working Party of Principal Laboratory Officers, first published by the Australian Bureau of Animal Health (1978).

Reprinted by CSIRO for the Australian Agricultural Council (1987) as: Australian Standard Diagnostic Techniques for Animal Diseases, No. 3, *Brucellosis – Serum Agglutination Test*.

*Standard ELISA Test for Bovine Brucellosis*, by P. Plackett and J. Stewart and first published by CSIRO for the Australian Agricultural Council (1986) as: Australian Standard Diagnostic Techniques for Animal Diseases, No. 6, *Standard ELISA test for Bovine Brucellosis*.

## 1. Introduction

Australia commenced the national campaign to eradicate bovine brucellosis in 1970, although control measures had been in operation already for several decades. Australia was declared free of bovine brucellosis in 1989. The control and eradication programs relied heavily on the detection of infected cattle by the identification of brucella-specific antibodies in serum.

*Brucella abortus* is the causative agent of bovine brucellosis. Contagious abortion, as it is commonly known, has a worldwide occurrence and was once one of the most serious diseases of dairy cattle in Australia where it caused considerable economic loss. This, together with the risk to the human population and the threat to our export trade in beef, were the prime motivations for the introduction of the national eradication campaign.

A definitive diagnosis of bovine brucellosis is achieved by the isolation of *B. abortus* from the foetus, uterine fluids or placenta from an aborting cow, or the uterus or udder and associated lymph nodes from an infected, non-pregnant cow. A presumptive diagnosis of bovine brucellosis is usually made by the application of one or more serological tests. The five serological tests used in the Australian eradication campaign are described below.

## 2. Brucella Milk Ring Test

### *2.1. General Information*

The Brucella Milk Ring Test (BMRT) is very sensitive, detecting the presence of positive milk from an infected cow when it has been diluted with milk from non-infected animals. The test is, therefore, extremely valuable as a presumptive or screening test on bulk or can samples to locate potentially infected herds, thereby reducing the number of serological tests needed for accrediting herds. It is also useful, if carried out regularly, for the assessment of progress in any eradication or control program.

The development of a positive reaction depends on the following two processes.

(a) A 'fat globule agglutinin' normally present in milk aggregates the fat globules which then rise to the surface on standing. This is the normal cream layer.

(b) Stained brucella cells which are added as antigen are agglutinated if brucella antibodies are present in the milk. These aggregated stained cells adhere to the surfaces of the fat globules and rise with them to form a blue-coloured cream layer.

#### 2.1.1. Brucella Milk Ring Test Antigen

The antigen is a haematoxylin stained suspension of killed *Brucella abortus* organisms. Phenol 0.5% w/v is added as preservative. The antigen is used to detect the presence of brucella antibodies in milk and cream. The antigen can be obtained from: FAO/WHO Collaborative Centre for Brucellosis, Central Veterinary Laboratory, New Haw, Weybridge, Surrey, UK).

#### 2.1.2. Precautions in Testing Milk

The following precautions should be kept in mind when testing milk.

(a) Incorrect sampling could lead to excessive or insufficient cream content. This will interfere with the reading of the test.

(b) Excessive shaking denatures the 'fat globule agglutinin' and upsets the formation of the surface cream layer.

(c) Heating at 45°C or above reduces the brucella antibody content.

(d) Milk may be stored at 2–5°C for up to two weeks without undue loss of reactivity. Longer periods and/or higher storage temperatures cause antibody loss. Frozen samples may give false reactions.

#### 2.1.3 False Positive Reactions

These may occur when the test is conducted on:

(a) freshly collected milk (the samples, therefore, must be refrigerated for at least 12 hours);

(b) milk from cows with mastitis;

(c) milk containing colostrum;

(d) milk from cows in the drying off period;

(e) milk from non-infected milking animals which have been vaccinated within the last three months with Strain 19 vaccine.

#### 2.1.4. Preservative

Bulk milk samples are preserved with Bronopol (2-bromo-2-nitropropane-1,3-diol; marketed as 'Myacide' by Boots Co. and distributed in Australia by Harcos Chemicals Pty Ltd, 3 Alan Rd, Rydalmere, NSW 2116. Tel. (02) 684 4122).

Add 200 µL of a 5% solution of 2-bromo-2-nitropropane-1,3-diol in water (containing 0.188% carmoisine edicol or eosin yellow as an indicator) to 25–40 mL of milk or cream. Mix gently but thoroughly and hold the sample at 2–5°C.

#### 2.1.5. Addition of Cream to Milk

Where the herd is composed of cows which produce milk of medium to high cream content, e.g. Jersey cows, it is not necessary to add extra cream to the test. Should the herd be one with a low fat production, e.g. Friesian, it may be necessary to add extra cream to the tests (0.1 mL of BMRT negative cream to each test).

##### 2.1.5.1. Pooled negative cream

Obtain untreated milk from a brucellosis-free herd containing at least 25 lactating cows. Add 1 mL of formaldehyde ($CH_2O$) to each litre of milk, mix and allow the cream to rise by storing in a refrigerator overnight. Remove the cream.

**Table 1.** Number of cows in the herd to be tested

| | |
|---|---|
| 1–4 lactating: | Test the pool (1 mL test). If positive, test the milk from each cow after dilution 1:10 in negative BMRT pooled milk. |
| 5–200: | Standard 1 mL test. |
| 201–500: | Use 2 mL of milk in the test. |
| 501–900: | Use 3 mL of milk in the test. |
| 901 or more: | Arrange for breakdown of herd so that not more than 120 lactating cows are represented in one pool. |

#### 2.1.6. Herd Size

The volume of milk used in the BMRT varies with the size of the herd.

These figures (more than five cows; Table 1) refer to total herd size not to lactating animals. The optimum number of lactating animals represented when a 1 mL BMRT is done is 30–120 cows.

### *2.2. Test Procedure*

#### 2.2.1. Standard Brucella Milk Ring Test

A representative sample from each bulk tank or can is collected after ensuring that the contents are thoroughly mixed before sampling. The sample should be refrigerated at 2–5°C for at least 12 hours but preferably 48–72 hours before testing. Sour milk is unsuitable for testing.

On the day of test, remove the milk samples and the BMRT antigen from the refrigerator and hold at room temperature for one hour before testing.

Mix each sample gently but thoroughly to ensure even dispersion of the cream. For each sample, place 1.0 mL of milk in a tube having a diameter of about 10 mm (3/8 inch) to give a milk column of about 2 cm. Add one drop (30 µL) of the BMRT Antigen to each tube (hold delivery end of pipette vertically). Mix the contents of each tube gently but thoroughly within one minute of the antigen addition.

Place the tubes vertically in a rack in a constant temperature water bath at 37°C for one hour.

#### 2.2.2. 2 mL and 3 mL Brucella Milk Ring Test

The procedure is as detailed above except that 2 or 3 mL of milk are used according to herd size. The antigen volume (30 µL) is unaltered.

The incubation period is the same. Read and record the results as shown in Table 2.

### *2.3. Interpretation*

Any degree of positive reaction is presumptive evidence of the presence of one or more infected animals in the herd. In a large herd, the presence of a very small number of infected animals may not be detected by the above technique unless their milk antibody levels are very high. It is recommended, therefore, that each herd be retested at intervals of one to three months. This procedure also includes cows which were not lactating at the time of previous testing.

### *2.4. Individual Animal Brucella Milk Ring Test*

When positive BMRT results in the herd test are not confirmed by herd blood tests, it is useful to examine milk from individual animals in the herd. Any positive milk from an animal should be retested after diluting 1 in 10 in a pool of several negative milks (1 mL diluted milk + 30 µL antigen). A positive result in this test is indicative of infection.

### *2.5. Cream Brucella Milk Ring Test*

Cream, as such, should not be tested by the standard BMRT. The following modification gives good sensitivity in detecting infected herds from which cream is produced. Sour cream is unsuitable for testing and results from cream that sours during testing should be disregarded.

The test is adversely affected by:

(a) pH below 5.0; and
(b) collection and storage periods which, when combined, exceed two weeks — this time is influenced by storage conditions and general hygiene.

#### 2.5.1. Reagents

##### 2.5.1.1. Precollection diluent

| | |
|---|---|
| Saturated sodium bicarbonate solution, $NaH_2CO_3$ | 20 mL |
| Formalin (37–40% $CH_2O$) | 1 mL |
| Water | 160 mL |

**Table 2.** Interpretation of the Brucella Milk Ring Test

| *Cream ring* | *Milk column* | | *Reaction* | *Test result* |
|---|---|---|---|---|
| | *No eosin* | *Eosin in preservative* | | |
| Intensively coloured blue | White | Pink | ++++ | Positive |
| Definitely coloured | Slightly coloured | Slight blue tone | +++ | Positive |
| Definitely coloured | Moderately coloured | Moderate blue tone | ++ | Positive |
| Moderately coloured | Moderately coloured | Moderate blue tone | + | Positive |
| White or slightly coloured | Definitely coloured | Definite blue tone | – | Negative |

2.5.1.2. Modified cream ring test neutraliser

(a) NaCl 8.5 g
$H_2O$ 1 L
Dissolve the sodium chloride in the water and add sodium bicarbonate salt to give a saturated solution.

(b) Evaporated milk.

Mix equal parts of (a) and (b) as required. BMRT negative cream is pooled from at least 20 cows.

2.5.1.3. Brucella milk ring test antigen

2.5.2. Test Procedure

(a) Mix each cream sample gently but thoroughly.
(b) Place 0.6 mL precollection diluent (see 2.5.1.1.) in a centrifuge tube and add 4 mL cream. Mix.
(c) Refrigerate (2–5°C) until testing starts.
(d) At test time remove sample from refrigeration and warm to room temperature.
(e) Centrifuge each cream sample at about 1000 *g* for 15 min.
(f) Observe after centrifugation. Those which appear white (like skim milk) are classified as not sour. Those appearing colourless to slightly yellow are classified as sour and should be discarded.
(g) Remove 1.2 mL from the liquid layer. Avoid fat and milk solids as these interfere with the test. The liquid may be removed by using a needle or cannula, e.g. 15G x 4' and a 2 mL syringe.
(h) Add the 1.2 mL of liquid to 0.6 mL of the 'modified cream ring test neutraliser solution'(see 2.5.1.2.) in a 14 x 100 mm tube. Mix gently but thoroughly.
(i) Add 0.4 mL fresh BMRT negative raw cream. This cream should be a pool from at least 20 cows.
(j) Add 1 drop (30 µL) BMRT antigen and mix.
(k) Incubate in a waterbath for one hour at 37°C.
(l) Observe and record the results as for the BMRT.
(m) Interpret the test as for the milk BMRT.

## 3. Complement Fixation Test

### *3.1. Introduction*

The technique of the Complement Fixation (CF) Test is modelled on that described in Anonymous (1965).

Titrations are carried out in tubes and the total volume is normally 1 mL (macrovolume). The diagnostic test is carried out in disposable plastic plates in volumes one-tenth of those used in the titrations (microvolumes). Various degrees of automation may be applied to this system. Warm fixation was chosen mainly for convenience. The 'prozone' problems that occur in this test need to be taken into account.

The system is extremely sensitive; the complement titration is capable of detecting much smaller differences in complement activity than are methods using 100% haemolysis as end point. To avoid differences in sensitivity between tests, great care is necessary in handling the various components, especially complement and erythrocyte suspension, and in the preparation of glassware used for titrations. When this care is taken, excellent reproducibility of results may be expected in the diagnostic tests. The titrations may at first sight appear complicated, but after a little practice they are rapidly and easily performed.

All glassware must be chemically clean, and preferably sterile. After use, glassware should be cleaned in detergent solution to remove all reagents and then placed in dilute chromic acid cleaning fluid for 18 hours [dilute chromic acid cleaning fluid comprises potassium dichromate ($K_2Cr_2O_7$), 20 g; sulfuric acid ($H_2SO_4$), 76 mL; distilled water to 1 L] or in a commercial preparation (such as RBS 25 or Decon) designed to serve the same purpose. It should then be rinsed thoroughly in tap water before finally being rinsed twice in distilled water. Glassware should be dried in the hot air oven.

### *3.2 Materials*

3.2.1. Diluent

Barbital buffered salt solution is used in preparing all solutions and suspensions in the standardised CF test. The following method of preparation is taken from Kabat and Mayer (1961).

(a) Prepare a stock solution containing 1 mol/L magnesium chloride ($MgCl_2.6H_2O$) and 0.3 mol/L calcium chloride ($CaCl_2.2H_2O$), e.g. 20.33 g of magnesium chloride hexahydrate and 4.41 g of calcium chloride dihydrate made up to 100 mL in distilled water. Filter sterilise and store in the refrigerator in small amounts.
(b) Dissolve 85 g of sodium chloride and 3.75 g of sodium 5.5-diethyl barbiturate (barbital sodium, $C_8H_{11}N_2O_3Na$) in about 1.4 L of distilled water.
(c) Dissolve 5.75 g of 5.5-diethyl barbituric acid (barbital, $C_8H_{12}N_2O_3$) in about 500 mL of hot distilled water.
(d) Mix the solutions prepared in (b) and (c), cool to room temperature, add 5 mL of the magnesium and calcium stock solution described in (a), add distilled water to make a final volume of 2 L. This is the concentrated barbital buffer solution, which should be stored in a refrigerator.
(e) For use, one part of the concentrated buffer solution is mixed with four parts of cold distilled water. It is kept in the refrigerator until required. Freshly diluted buffer should be prepared each day. The pH of the diluted buffer should be 7.3–7.4. Some workers prefer to dilute the concentrated

buffer in four parts of sterile 0.04% gelatin solution rather than in distilled water. Tablets may be used for preparing barbital buffered salt solution.

Kolmer diluent, the formula for which is given in 8.1. may be used as an alternative.

### 3.2.2. Collection and Storage of Sheep Blood

Sheep known to produce erythrocytes of a consistently satisfactory level of sensitivity should be chosen and used exclusively. Blood is withdrawn under aseptic conditions into an equal volume of Alsever's Solution (see 8.2.) and thoroughly mixed. The sheep blood so preserved is stored aseptically in screw-capped bottles in the refrigerator and should not be used until at least five days after collection; thereafter, it may be used for up to one month. Sheep red blood cells in Alsever's solution are available commercially from: Filtrona Pty Ltd, PO Box 425, Altona North, Vic. 3205, Australia.

### 3.2.3. Washing the Erythrocytes

Up to 10 mL of the sheep blood stored in Alsever's solution is placed in a 50 mL centrifuge tube which is filled with diluent and the contents thoroughly mixed. The suspension is centrifuged to sediment the erythrocytes and the supernatant discarded along with the buffy coat, i.e., the thin layer of white cells that overlays the deposit. The erythrocytes are suspended in fresh diluent and the centrifugation repeated. For the third and final centrifugation the erythrocytes are resuspended in about 15 mL of diluent and centrifuged in a graduated tube at about 1000 *g* for 10 min. The deposit is used to prepare the suspension.

### 3.2.4. Standardisation of the Erythrocyte Suspension

The standardised erythrocyte suspension used should contain 0.95 g haemoglobin per 100 mL as determined by the cyanmethaemoglobin method described in 8.3. which is equal to a suspension containing $6x10^8$ erythrocytes as determined in an electronic cell counter. Such a suspension is equal to a 3% suspension of erythrocytes obtained by centrifuging a suspension of sheep erythrocytes at exactly 1000 g for 10 min in a graduated centrifuge tube and resuspending the deposit in 32.3x its volume of diluent.

In routine work the erythrocyte suspension is adjusted by using a spectrophotometer as described below, so that when 1 mL of the erythrocyte suspension is haemolysed in 15 mL of distilled water it will produce the required optical density (OD) on the machine; this is known as the target OD. The target OD for the particular machine being used is determined as described in 8.3.

### 3.2.5. Routine Preparation of the Standardised Erythrocyte Suspension

The deposit after the third centrifugation (see 3.2.3.) is suspended in about 27x its volume of diluent to make an erythrocyte suspension somewhat denser than that required for the standardised suspension; after thorough mixing, 1 mL of this suspension is haemolysed by mixing with 15 mL of distilled water and its OD determined.

The amount of diluent that needs to be added to the denser suspension to produce a suspension of the required density is calculated according to the formula:

Amount of diluent to be added (mL)=
(OD of denser suspension – Target OD)/
Target OD x No. mL to be diluted

e.g. when the OD of the denser suspension = 0.61, the target OD = 0.5 and the No. of mL of the denser suspension is 25, then the No. of mL of diluent to add is:

[(0.61 – 0.5)/0.5 x 25] or
[(0.11/0.5) x 25] = 5.5 mL

Each final suspension must be checked before use by haemolysing a sample (1 mL in 15 mL of distilled water) and verifying that the resulting suspension gives the target OD.

As an alternative, the standardised erythrocyte suspension may be prepared by carrying out the third centrifugation (see 3.2.3.) at exactly 1000 *g* for 10 min then suspending the deposit in 32.3x its volume of diluent. In this case the resulting suspension should be checked regularly, at least once a month, by determining the concentration of haemoglobin per 100 mL using the cyanmethaemoglobin method.

The standardised erythrocyte suspension if stored at about 4°C may be used for up to 24 hours after preparation or for a longer period provided there is no evidence of lysis in the suspension.

### 3.2.6. Haemolysin (Amboceptor)

The haemolysin is titrated to determine the concentration which, when added to an equal volume of standardised erythrocyte suspension, will produce an optimally sensitised erythrocyte suspension.

The haemolysin should be prepared in rabbits. Details of the technique of producing haemolysin may be found in most text books giving serological techniques, e.g. Campbell *et al.* (1970) or Cruickshank *et al.* (1973). Haemolysin is also available commercially usually in liquid form preserved with an equal quantity of glycerine. (Haemolysin is available in Australia from: Edward Keller Aust., Pty Ltd, Medical Division, Private Bag 3, Mordialloc, Vic. 3195; and Gilles Plains Animal Resource Centre, 101 Blacks Rd, Gilles Plains, SA 5086.) Haemolysin preparations of less than a titre of 1:500 should not be used.

In use, a 1:100 stock dilution of glycerinated haemolysin may be made in CF diluent and stored in frozen aliquots. The haemolysin is titrated each time a batch of 1:100 dilution is made and each time a new batch of erythrocytes is brought into use.

3.2.7. Titration of the Haemolysin

(a) Prepare the standardised erythrocyte suspension as already described (see 3.2.2.–3.2.5.).

(b) From the 1:100 stock dilution of haemolysin prepare the following range of dilutions in CF diluent: 1 in 500, 1000, 1500, 2000, 3000, 5000 and 10 000. For example:
Add 1 mL 1:100 dilution of haemolysin to 4 mL diluent to give a 1:500 dilution
Add 1 mL 1:100 dilution of haemolysin to 9 mL diluent to give a 1:1000 dilution
Add 2 mL 1:1000 dilution of haemolysin to 1 mL diluent to give a 1:1500 dilution
Add 1 mL 1:1000 dilution of haemolysin to 1 mL diluent to give a 1:2000 dilution
Add 1 mL 1:1000 dilution of haemolysin to 2 mL diluent to give a 1:3000 dilution
Add 1 mL 1:1000 dilution of haemolysin to 4 mL diluent to give a 1:5000 dilution
Add 1 mL 1:1000 dilution of haemolysin to 9 mL diluent to give a 1:10 000 dilution.

(c) Add 1 mL of each haemolysin dilution to 1 mL of standardised erythrocyte suspension while gently agitating the erythrocyte suspension, then leave the mixtures at 37°C for 15 min to allow sensitisation of the erythrocytes to occur, agitating the tubes every five minutes.
The rest of the procedure is done in duplicate to minimise pipetting errors, the tubes being kept cold, while the reagents are being dispensed, e.g. stood in a polystyrene foam container containing crushed ice.

(d) To each of a duplicate series of seven tubes add 1.0 mL of diluent and 0.5 mL of complement diluted in such a way that it will produce about 70–80% haemolysis with the more concentrated haemolysin dilutions (with good quality complements a 1:350 dilution is satisfactory).

(e) From each of the haemolysin–erythrocyte mixtures in turn 0.5 mL is transferred to each of a pair of tubes containing complement and diluent and thoroughly mixed.

(f) Incubate the tubes for 30 min in a water bath at 37°C with gentle shaking after 15 min.

(g) Remove the tubes from the water bath, add 2 mL of cold diluent to each, then centrifuge the tubes to deposit any erythrocytes remaining unlysed.

(h) Pour off the supernatant from each tube and read the ODs in a spectrophotometer. This results in duplicate readings being obtained for each haemolysin dilution tested.

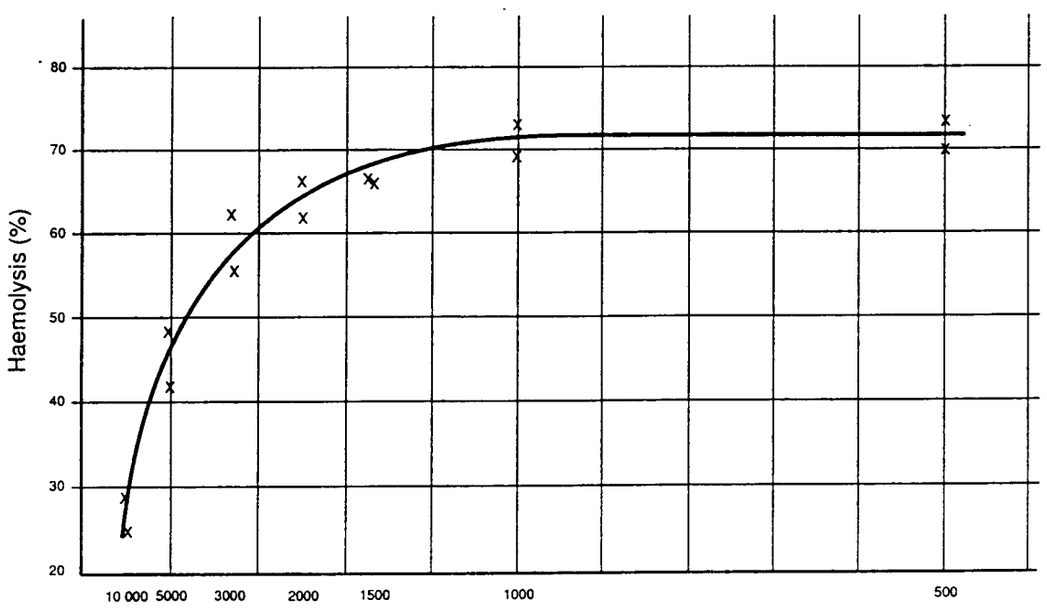


**Figure 1.** Titration of the haemolysin.

(i) The OD given by 100% haemolysis is the same as the target OD used in preparing the standardised erythrocyte suspension.

(j) Calculate the percentage of haemolysis for each tube in the titration, e.g. supposing the OD produced by 100% lysis is 0.5, a tube showing an OD of 0.21 would have 0.21x 100/0.5 = 42% haemolysis.

(k) Plot the percentage haemolysis given by each dilution on graph paper marked out as shown in Fig. 1. To calibrate the abscissa, measure an arbitrary distance, say 10 scale divisions, from the left hand extremity and place a point here representing the 500 dilution; this is the extreme right-hand end of the abscissa. Distances from the left-hand end for the points representing the other dilutions are calculated by dividing the reciprocal of the dilution in 500, e.g. the point for the 1000 dilution is placed 500/1000 or half way along the line, the 5000 dilution 500/5000 or one-tenth of the way along etc. The percentage haemolysis is marked linearly along the ordinate, the points for percentage haemolysis are plotted for each haemolysin dilution and the line drawn, ignoring outlying points.

(l) Optimal dilution of haemolysin for use in the test is decided by determining where the plateau begins (1:1000 in Fig. 1) and selecting a dilution about 25% more concentrated (1:800 in the example given) for use in the test. The selection of the quantity of haemolysin to use in the test is not critical so long as an ample amount is chosen. The quantity of haemolysin forms a fixed point against which the amount of complement to be used is determined accurately.

### 3.2.8. Complement

At least four guinea pigs should be bled, the serum separated as soon as practicable from the clot and pooled to produce complement. Adult guinea pigs receiving adequate green food produce good quality complement but all food should be withheld during the 12 hours preceding bleeding; pregnant females or those that have recently given birth should not be used.

Complement may be preserved by Richardson's method and when so preserved will maintain its titre for about six months if stored at 4°C. Even at room temperature the loss of titre is not rapid.

Two stock solutions which keep indefinitely are used:

(a) Solution A

| | |
|---|---|
| Boric acid, $H_3BO_3$ | 0.93 g |
| Borax, $Na_2B_4O_7.10H_2O$ | 2.29 g |
| Sorbitol, $C_6H_{14}O_6.1/2\ H_2O$ | 11.74 g |
| Saturated NaCl solution to | 100 mL |

(b) Solution B

| | |
|---|---|
| Borax | 0.57 g |
| Sodium azide, $NaN_3$ | 0.81 g |
| Saturated NaCl solution to | 100 mL |

To preserve complement, mix eight parts of guinea pig serum with one part of (b), followed by 1 part of (a). Before use it is necessary to restore tonicity by adding one part of preserved complement to seven parts of distilled water. This gives a 1:10 dilution of complement.

Unpreserved complement may be stored frozen at -70°C or below, storage in liquid nitrogen is satisfactory, or in the dried state. Dried complement is also available commercially in Australia from: ICN Biomedicals, Unit 12/31 Sevenhills Road, North Sevenhills, NSW 2247; and Gilles Plains Animal Resource Centre, 101 Blacks Rd, Gilles Plains, SA 5086. Complement should be stored in the refrigerator or freezer.

### 3.2.9. Titration of Complement

Complement is titrated: (a) before using a new batch of complement; and (b) before using a new collection of sheep blood to prepare the erythrocyte suspension. The quantity of complement required to lyse 50% of optimally sensitised erythrocytes is determined, this is called 1 $C'H_{50}$; 5 $C'H_{50}$ are used in the test. A master dilution of complement is prepared. When complement preserved by Richardson's method is being used this will be the 1:10 dilution produced by reconstitution in distilled water. The master dilution should be made in fluid at refrigerator temperature (2–5°C) and stored at this temperature. A small quantity of the master dilution is further diluted to produce the titration dilution; with good quality complement the titration dilution is likely to be 1:350 but this may have to be determined by trial.

The procedure for the titration is as follows:

(a) Prepare sensitised erythrocytes by mixing equal volumes of standardised erythrocyte suspension and haemolysin diluted as determined in 3.2.7. Allow the mixture to stand at 37°C for 15 min, mixing every five minutes.

**Table 3.** Complement titration — arrangement and quantities for tube titration

| | *Tubes* | | | | | |
|---|---|---|---|---|---|---|
| | *1* | *2* | *3* | *4* | *5* | *6* |
| Complement 1 in 350 mL | 0.3 | 0.4 | 0.5 | 0.6 | 0.7 | 0.8 |
| Diluent mL | 1.2 | 1.1 | 1.0 | 0.9 | 0.8 | 0.7 |
| Place in water bath at 37°C for 30 min then add | | | | | | |
| Sensitised erythrocytes mL | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |

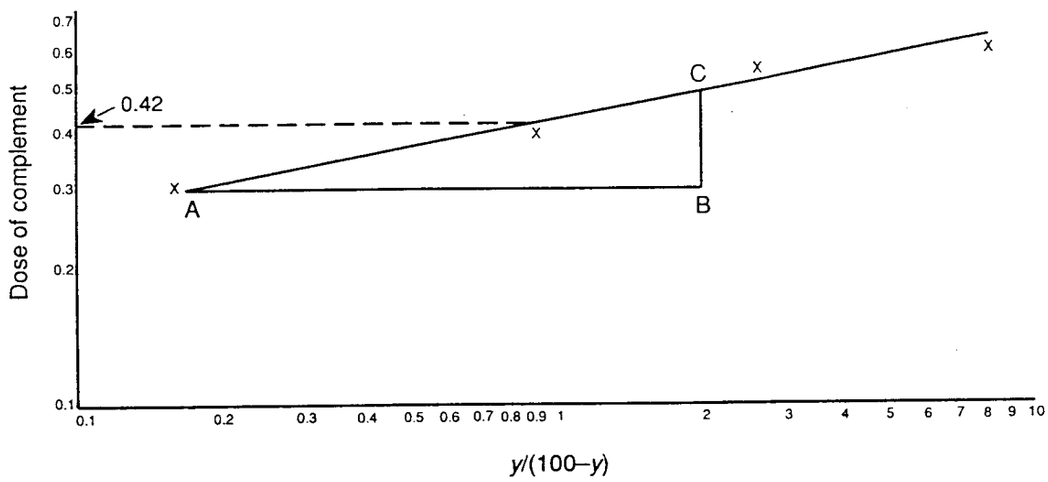


**Figure 2.** Calculation of the dose of complement to produce 50% haemolysis.

(b) Prepare master and titration dilutions of complement.

(c) This procedure is done in duplicate to minimise pipetting errors. The test is done in twice the standard volume to minimise errors in pipetting and to produce a total volume large enough to give a reading on the spectrophotometer. Arrange six suitable tubes in a rack and add the reagents in the quantities and the order shown in Table 3. After the diluent has been added the contents are mixed thoroughly and the tubes are placed in a water bath at 37°C for 30 min.

(d) After the addition of the sensitised erythrocytes the contents of each tube are mixed by gentle agitation then incubated in a water bath at 37°C for 30 min, during which time they are agitated at least once. After the incubation is completed the tubes are removed from the water bath, 2.0 mL of cold diluent is added to each tube and the tubes centrifuged to deposit the unlysed cells. The supernatants are then poured off and their ODs determined in the spectrophotometer.

Supposing the OD corresponding to 100% lysis is 0.5, a reading of 0.2 would indicate:

$$[(0.2 \times 100)/0.5] = 40\% \text{ haemolysis}$$

**Table 4.** Calculation of the percentage of haemolysis in complement titration

| *Dose of complement* | *Optical density (OD)* | *Percentage haemolysis (y)* | *y /(100-y)* |
|---|---|---|---|
| 0.3 | 0.06 | 13 | 13/87= 0.15 |
| 0.4 | 0.22 | 47 | 47/53 = 0.89 |
| 0.5 | 0.34 | 72 | 72/28 = 2.6 |
| 0.6 | 0.42 | 89 | 89/11 = 8.1 |

(e) The dose of complement required to produce 50% haemolysis may now be determined graphically by plotting the degree of haemolysis for each dose of complement used in the titration on log/log paper, i.e. paper with log scales both vertically and horizontally (Fig. 2). The degree of haemolysis is calculated for each tube from the expression $[y/(100\text{-}y)]$ where $y$ is the percentage haemolysis (only tubes showing between 10 and 90% haemolysis are taken into consideration).

(f) The values for the expression $[y/(100\text{-}y)]$ are plotted for each dose of complement (Fig. 2) and a straight line fitted through the points. The point where the line 'AC' has a value of 1 on the abscissa indicates the 50% haemolytic dose ($C'H_{50}$) and this may be read from the ordinate (broken line in Fig. 2). The calculation is made as in the example given in Table 4.

(g) A simple calculation suffices to arrive at the dilution factor required for the diagnostic test. In our example 0.42 mL of a 1:350 dilution contains 1 $C'H_{50}$; therefore 5x 0.42, i.e. 2.1 mL of this dilution will contain five $C'H_{50}$ and to calculate the dilution that will contain this amount of complement in 0.5 mL (here we are using double volumes) the following equation is used wherein $x$ is the dilution factor required:

$$350/2.1 = x/0.5; \text{ i.e. } x = 83.$$

This formula may be simplified to:
$x$ (dilution factor) =[titration dilution of complement/10x50% haemolytic dose]
i.e. in our example: 350/4.2 = 83
Smaller volumes being used in the diagnostic test does not affect this dilution factor since

the proportions of each reagent remain the same. The 1/10 dilution of complement now being stored in the refrigerator needs, therefore, to be diluted 83/10, i.e. 8.3x, to bring it to the 1:83 required for use in the diagnostic test, i.e. each 1 mL of the 1:10 dilution is added to 7.3 mL of diluent. The 1:10 dilution will maintain its potency throughout the working day if stored as described.

(h) To save repeated calculations a table of the percentage haemolysis and $[(y/100)\text{-}y]$ for each spectrophotometer reading should be drawn up (see 8.4.).

(i) The validity of the slope of the line is now examined. A horizontal line 10 cm long (line AB in Fig. 2) is drawn with its left hand extremity touching the slope at a convenient point. From the right hand end of this horizontal line at B a vertical line is extended up to the point C where it meets the slope. With a satisfactory slope the length of the vertical line should be one-fifth of the length of the horizontal line ± 10%, i.e. not more than 22 mm and not less than 18 mm long. Though complement giving slopes steeper or flatter may give satisfactory results in the diagnostic test, experience has shown that accurate and reproducible results are more likely to be obtained if only complements satisfying these criteria are used.

### 3.2.10. Antigen

The antigen for the CF test is available from: FAO/WHO Collaborative Centre for Brucellosis, Central Veterinary Laboratory, New Haw, Weybridge, Surrey, UK. The antigen should be diluted freshly for each day's test.

### 3.2.11. Inactivation of Sera

In addition to the natural anticomplementary activity present in serum, certain conditions, particularly bacterial contamination, may produce an added degree of anticomplementary activity. Haemolysis in serum has little, if any anticomplementary effect. The anticomplimentary activity due to bacteria may be greatly reduced by centrifuging the serum at 8000 *g* or greater for 15 min (or equivalent). Other methods of removing anticomplementary activity are available.

The natural anticomplementary activity in bovine serum is removed by heating at 58°C for 30 min in a water bath. Serum may also be inactivated in the trays (see 3.3.) in an incubator at 58°C. If this is to be successful it is necessary to ensure that: (a) the plates are not stacked more than three high; (b) the incubator door is not opened during inactivation; and (c) inactivation begins when the temperature of the incubator regains 58°C — a fan installed in the incubator aids the recovery of the operating temperature after the door has been opened.

### *3.3. Test Procedure*

The test is carried out in disposable plastic microtitre plates with U-shaped wells. The volumes of reagents used are one-tenth of those used in the titrations. Owing to the frequent occurrence of 'prozones', serum dilutions up to at least 1:128 should be tested. Normally 12 sera are tested on each plate; this provides eight wells per serum.

For automated systems the following procedure is recommended. A convenient amount of undiluted serum (at least 50 µL) is placed in the first well of the row, the wells containing serum are covered with tape and inactivated in an incubator at 58°C for 30 min. After inactivation, 25 µL of diluent is added to each well except the first of each row, doubling dilutions are made mechanically, starting with the undiluted serum and continuing right across the plate. Antigen (25 µL) is added to each well from the third to the end of each row. The second well acts as an anticomplementary control and requires the addition of a further 25 µL of diluent to compensate for lack of antigen. Complement (25 µL) is added to each well except the first of each row. (The test is read from the 1:4 dilution, i.e. the third well, onwards; that the anticomplementary control is more concentrated than the first dilution of the test proper is not considered to have practical significance. Sera judged as anticomplementary in this system can be retested manually).

Antigen and complement may be mixed and added together. The reagents are mixed by tapping the plate gently, the plates are covered, e.g. by another plate, and placed in an incubator at 37°C for 30 min. The sensitised erythrocyte suspension is prepared by mixing equal volumes of standardised erythrocyte suspension and haemolysin dilution and keeping the mixture at 37°C for 15 min, agitating gently every three to five minutes. After the plates are removed from the incubator 25 µL of sensitised erythrocyte suspension is added to each well except the first of each row. It is important that the sensitised erythrocytes are thoroughly mixed with the other components and kept in suspension; this can be best achieved by using a mechanical shaker installed in the 37°C incubator. After shaking for a further 30 min at 37°C the plates are removed from the incubator. They are either centrifuged at 2000 *g* for 10 min in the special microtitre carrier plates or placed in the refrigerator for at least two to three hours to ensure deposition of any unlysed erythrocytes. The results can now be read; this may be done over a white diffused light or from below using a magnifying mirror.

### *3.4. Interpretation*

When the reaction in any well is negative, the contents are completely clear and pink due to haemoglobin released from the lysed cells. Complete fixation in the absence of haemaggluti-

**Table 5.** Standards for agglutination tests

| | *Degree of agglutination* | | | | |
|---|---|---|---|---|---|
| | ++++ | +++ | ++ | + | – |
| Phenol-saline (mL) | 1 | 0.75 | 0.5 | 0.25 | 0 |
| Antigen, diluted 1:2 (mL) | 0 | 0.25 | 0.5 | 0.75 | 1.0 |
| Simulated percentage clearing | 100 | 75 | 50 | 25 | 0 |

nation appears as a compact button of erythrocytes in the centre of the well surrounded by clear, colourless fluid; lesser degrees of fixation produce smaller buttons of erythrocytes and pink fluid. When agglutination of unlysed erythrocytes occurs, and this is uncommon beyond the 1:8 dilution, there is a film of erythrocytes covering the whole of the bottom of the well. This may be a very fine film when the degree of fixation is only slight; these are nevertheless regarded as positive reactions of varying degrees.

A control serum of moderate titre (say 1:16) is tested in each row of a plate. Such a control plate is included with each set of tests or after every 20 plates.

A complement control may be set up. Working dilution (50 µL) of complement and 0.7 mL of diluent are placed in each of four tubes. These are incubated for 30 min in the water bath at 37°C, after which 0.25 mL of sensitised erythrocytes are added. After a further 30 min in the water bath, 1 mL of cold diluent is added, the tubes centrifuged and the average percentage haemolysis of the supernatants determined. This average should represent between 25 and 75% haemolysis.

## 4. Serum Agglutination Test

### *4.1. Introduction*

The serum agglutination test (SAT) used in Australia is the European Tube Agglutination Test described by Alton *et al.* (1988). The technique for carrying out the test is described in 4.2. Details of the production and standardisation of the antigen may be found in the original publication.

### *4.2. Test Procedure*

The tests may be done in either glass or plastic tubes suitable for working with 1 mL volumes. In view of the occasional occurrence of prozone phenomena, at least five tubes are normally used for each serum under test. Using an automatic pipette for preference, 0.8 mL of phenol–saline (0.85% sodium chloride and 0.5% phenol) is placed in the first tube and 0.5 mL in each succeeding tube; 0.2 mL of the serum under test is transferred to the first tube and mixed thoroughly with the phenol–saline already there; 0.5 mL of the mixture is carried over to the second tube from which, after mixing 0.5 mL is transferred to the third tube, and so on. This process is continued until the last tube, from which, after mixing, 0.5 mL of the serum dilution is discarded. This process of doubling dilutions results in 0.5 mL of dilutions 1:5, 1:10, 1:20 and so on, in each tube. To each tube is then added 0.5 mL of antigen at the recommended dilution and the contents of the tube are thoroughly mixed, thus giving final serum dilutions of 1:10, 1:20, etc. The tubes are then incubated at 37°C for 20 ± 1 hour before the results are read. The dispensing, mixing, and transferring of the serum under test may be done with a pipette, but these operations are more conveniently carried out with a 1 mL tuberculin syringe fitted with a needle that has had its beveled tip removed so that the tip just fails to reach the bottom of the tube. The hypodermic needle may be replaced by fine polythene tubing (Intramedic PE 50) fitted into the nozzle of the syringe and cemented in place; the 'dead space' with this type of tubing is negligible.

The degree of agglutination is assessed on the amount of clearing that has taken place in the tube as compared with a standard tube. The tubes are examined without being shaken against a black background with a source of light coming from above and behind the tubes. Complete agglutination and sedimentation with water-clear supernatant is recorded as ++++, nearly complete agglutination and 75% clearing as +++, marked agglutination and 50% clearing as ++, some sedimentation and 25% clearing as +, and no clearing as –.

The accuracy and reliability of the readings are much improved if standard tubes simulating degrees of agglutination are available for comparison. Standards should be prepared at the time the tests are done and incubated with them. The antigen is diluted by mixing 2 mL of antigen, diluted as for the test, with 2 mL of phenol–saline; the five standard tubes are prepared by mixing the quantities shown in Table 5.

### *4.3. Interpretation*

The results of the agglutination tests should be expressed in international units (IU) (see 4.4.), and interpreted according to the recommendations contained in the fifth report of the FAO/WHO Expert Committee of Brucellosis (FAO/WHO, 1971), which recommends that in cattle the minimum diagnostic level be 100 IU/mL for cattle ≤ 8 months of age and not vaccinated with strain 19. Levels 50% lower than these, i.e., 50 IU/mL for non-vaccinated and 100 IU/mL for vaccinated animals, should be regarded as 'doubtful' or 'suspicious'. Such animals should be retested after 60 days. Supplementary diagnostic tests are often helpful in deciding the status of animals that are classified as doubtful by agglutination tests.

### 4.4. The International Unitage System

By definition the International Standard anti-*Brucella abortus* serum (ISAbS) contains 1000 IU per ampoule. Therefore, using an antigen that gives a titre of 1:500 with the ISAbS, a serum under test giving a titre of 1:40 contains 1000x 40/500 = 80 IU/mL. Table 6 shows the conversion of titres to IU/mL.

## 5. Rose Bengal Test

### 5.1. Introduction

The Rose Bengal test (RBT) is a spot agglutination test used to screen herds. For the diagnosis of individual cattle the test is over sensitive, especially in cattle vaccinated with strain 19.

### 5.2. Materials

The test should be done on clear glass or plastic plates, marked with 15 mm squares delineated by araldite, or in WHO haemagglutination trays or equivalent.

All glass plates or plastic trays should be perfectly clean. After use, rinse immediately under the tap to remove all obvious residues. Soak in suitable detergent solution (RBS 25 or Decon) for at least two hours (preferably overnight). Rinse thoroughly in tap water and finally rinse twice in distilled water. Then dry in incubator or drying chamber (such as commercial clothes dryer).

The RBT antigen is obtained from the Central Veterinary Laboratory, Weybridge (see 3.2.10.).

### 5.3. Test Procedure

Serum samples and RBT antigen should be at room temperature. Because not all laboratories are air conditioned, no upper limit can be set for conducting the test, but around 20°C is desirable. The temperature should not be below 15°C.

Dispense serum samples in drops with an Eppendorf type pipette with a disposable tip. The volume dispensed to be 30 µL for the flat glass or plastic plates and 25 µL for the WHO haemagglutination trays.

Use the antigen at the concentration recommended by the manufacturers. Dispense the antigen with a dropping pipette. The volume must be equal to that of the serum sample.

To minimise delay between the adding of antigen to the first serum sample and the last serum sample, no more than one plate or tray per operator should be set up at the one time. The commencement time should be marked on the plate.

Immediately after the addition of the last drop of antigen to a plate or tray, each test should be mixed thoroughly by the method found most convenient in the testing laboratory.

With WHO trays, the following procedure is satisfactory:

Place the tray on a flat surface and hold squarely with both hands. Mixing of the tests is achieved by moving the hands first in a clockwise direction and then anticlockwise with increasing vigour, taking care to avoid spillage. This results in a swirling effect and the test mixture must cover the whole of the bottom of the well. With flat trays, mixing may be by gentle rocking and tapping of the trays, or by stirring with clean toothpicks. Mixing should be carried out over a template so that an adequate *uniform* area is covered by the test mixture. After mixing, the plate or tray is immediately placed on a rocker.

The test plate should be rocked mechanically for four minutes at a rate of about 30 oscillations per minute. The tests should then be read without delay. When reading the tests the plate should be slowly tilted back and forth over a light source of even intensity which may be indirect. A suitable system of double checking the readings should be used to reduce or eliminate operator error.

### 5.4. Interpretation

A scoring system for results should be used, for correlation with CF test results. The following allows distinction of degree of reaction:

0 — No agglutination, no rimming, uniform pink colour;

1 — Barely perceptible agglutination and/or some rimming;

2 — Fine agglutination, definite rimming, Some clearing;

3 — Coarse clumping, definite clearing.

Samples giving reactions of 1, 2 or 3 should be subjected to the CF test.

**Table 6.** Conversion of titres to International Units/mL for an antigen giving a titre of 1:500 with the International Standard Anti-*Brucella abortus* Serum (ISAbS)

| *Final dilution of serum* | *End point reading* | *IU/mL* |
|---|---|---|
| 1/10 | 1+ | 17 |
| | 2+ | 20 |
| | 3+ | 23 |
| | 4+ | 27 |
| 1/20 | 1+ | 34 |
| | 2+ | 40 |
| | 3+ | 47 |
| | 4+ | 53 |
| 1/40 | 1+ | 67 |
| | 2+ | 80 |
| | 3+ | 93 |
| | 4+ | 106 |
| 1/80 | 1+ | 134 |
| | 2+ | 160 |
| | 3+ | 186 |
| | 4+ | 212 |
| 1/160 | 1+ | 268 |
| | 2+ | 320 |
| | 3+ | 372 |
| | 4+ | 424 |
| 1/320 | 1+ | 536 |
| | 2+ | 640 |
| | 3+ | 744 |
| | 4+ | 848 |
| 1/640 | 1+ | 1072 |
| | 2+ | 1280 |
| | 3+ | 1488 |
| | 4+ | 1696 |

### *5.5. Standardisation*

The sensitivity of the test should be checked each day by putting up a standard working positive serum. The serum is available from: FAO/WHO Collaborative Centre for Brucellosis, Central Veterinary Laboratory, New Haw, Weybridge, Surrey, UK.

This serum should give a '3' reaction when undiluted and a '2' and '1' reaction at nominated dilutions. If the serum does not give these reactions, testing should not be proceeded with, and the cause of the incorrect reactions should be investigated.

The sensitivity of the batch of antigen can be checked against a battery of sera giving different degrees of reaction. The pH of the antigen should be 3.65 ± 0.095. The buffering capacity of the antigen can be checked by mixing one volume of antigen with one volume of freshly collected bovine serum. The pH should remain below 4.0.

## 6. Enzyme-Linked Immunosorbent Assay

### *6.1. Introduction*

The enzyme-linked immunosorbent assay (ELISA) for bovine brucellosis is a simple and sensitive assay for diagnostic use. Significant titres usually develop soon after initial infection and persist for the life of the animal.

Large changes in the relative proportions of the different isotypes occur during the course of a response but do not impinge on the performance or the interpretation of the assay. The optimum cut-off point for diagnosis of infection in unvaccinated cattle is about 64 N units/mL (i.e. a 32 000-fold dilution of the NSW standard serum) with commercial antibovine IgG (H + L chain) reagents.

### *6.2. Reagents*

#### 6.2.1. Phosphate Buffered Saline, pH 7.2

Phosphate buffered saline (PBS) is made up by mixing:

Sodium dihydrogen phosphate, $NaH_2PO_4.2H_2O$ 28 mL
0.2 mol/L (31.8 g/L)

Disodium hydrogen phosphate, $Na_2HPO_4.7H_2O$ 72 mL
2 mol/L (53.6 g/L)

and adding a solution of 8.0 g of sodium chloride in 900 mL of glass-distilled water.

The heptahydrate is specified because of its stability in air. The anhydrous salt is hygroscopic and will absorb two to seven moles of water per mole depending on temperature and humidity. The dodecahydrate readily loses five moles of water.

#### 6.2.2. PBS + Tween 20 (PBST)

PBST is made up from PBS with the addition of 0.5 mL of Tween 20 per litre.

#### 6.2.3. Antigen

The crude antigen, consisting mainly of lipopolysaccharide (LPS), is prepared from smooth *Brucella abortus* (strain 19) cells by the method of Berman *et al.* (1980). ELISA LPS *Brucella abortus* antigen is available from: Dr J. E. Searson, Regional Veterinary Laboratory, Department of Agriculture, Wagga Wagga, NSW, 2650, Australia. The product requires treatment with alkali before use. The extraction procedure and the method of alkali treatment are described in 8.5.

#### 6.2.4. Enzyme-labelled Antiglobulin Conjugates

Suitable preparations of antibovine IgG (H + L chains) or F(ab)2, conjugated to horseradish peroxidase (HRP) or to alkaline phosphatase (AP), are obtainable from commercial sources. Conjugates are stored at -10 to -15°C, after addition of an equal volume of glycerol to prevent freezing.

The optimum working dilution of each batch of conjugate is determined by checker-board titration against serial dilutions of the reference serum on an antigen-coated tray.

#### 6.2.5. Substrates

5-Aminosalicylic acid (5-AS, MW 153), o-phenylenediamine (oPD, MW 108), and 2,2'-[azinodi(3-ethylbenzthiaxoline sulfonate)] (ABTS) can all be used with HRP-conjugates. 5-AS can be conveniently purified and made up as a stock solution at 1 mg/mL and pH 6, by the method of Ellens and Gielkens (1980). It is less toxic than oPD (Sax, 1984) and is less likely to cause skin sensitivity. The stock solution, without added hydrogen peroxide, can be stored frozen in convenient amounts and thawed as required.

A 0.1 mol/L solution of hydrogen peroxide is prepared shortly before use by diluting 0.5 mL of 30% (8.8 mol/L) $H_2O_2$ solution (analytical reagent, AR grade) with 43.5 mL of distilled water. The concentration of peroxide solution is easily checked by measuring the absorbance at 240 nm, at which the molar absorbance is 43.6. Just before use, 0.2 mL of the 0.1 mol/L solution is added for each 10 mL of the stock 5-AS solution.

The preferred substrate for AP-conjugates is p-nitrophenyl phosphate, dissolved in 1 mol/L diethanolamine buffer pH 9.8, to a concentration of 1 mg/mL.

#### 6.2.6. Stopping Reagents

Under the usual assay conditions, the peroxidase-catalysed reaction is roughly linear for only a short period at room temperature. The decline in rate is faster if the peroxide concentration or the temperature is raised. Except for very short incubation times, the decline more than offsets any increase in the initial rate due to changes in these variables. After incubation for 30 min the

rate of reaction is low and the trays may be read without the addition of stopping reagents. The 5-AS reaction product is read at 450 nm.

Reactions catalysed by AP can be stopped by adding sodium hydroxide before reading the absorbency at 405 nm.

### 6.2.7. Reference Serum

The contents of an ampoule of freeze-dried NSW standard anti-*B. abortus* reference serum (batch Nos. 20083/20085) are reconstituted in 1.0 mL of pure water. (Batch Nos. 20083 and 20085 are available from: Dr J.E. Searson, Regional Veterinary Laboratory, Department of Agriculture, Wagga Wagga, NSW 2650, Australia.) This is then diluted 200-fold in PBST and 0.5 mL volumes are dispensed into screw cap plastic vials and stored at -20°C. For use, the contents of a vial are thawed and diluted to 5.0 mL in PBST. This solution, which is a 2000-fold dilution of the reconstituted serum, is assigned an antibody concentration of 1024 'N units'/mL.

### 6.2.8. Microtitre Trays

Some brands, including some marketed specially for ELISA work, show high levels of non-specific binding. Batch to batch, tray to tray and well to well variation are common. Several samples from any source should be tested before a particular batch is accepted for routine use. Brands found acceptable include the flat-bottomed Disposable Products ELISA tray and Linbro-Titertek catalogue No. 76-301-05 [available in Australia from Disposable Products, 810 Princes Hwy, Springvale, Vic. 3171. Tel. (03) 548 4411]. Some brands of trays with round-bottom wells may also be usable, in which case reagent volumes per well may be reduced.

### *6.3. Method*

(a) A solution containing 1 µg/mL of crude alkali-treated *B. abortus* LPS in PBS is added to the wells (100 µL for flat-bottomed wells and 50 µL/well for round-bottomed wells). Trays are covered with cellotape or with lids and shaken at 37°C for one hour, or kept overnight, with or without shaking, at 4°C.

(b) Antigen solution is shaken out and the trays are washed four times at room temperature. If automatic washing machines are not available, each wash is done as follows. The wells are filled with PBST and left for about one minute on the bench. Wash fluid is then shaken out and the operation is repeated. After the fourth wash, the trays are placed face down on clean filter paper and allowed to drain for 5–10 min.

(c) Serum dilution in PBST is placed in each of two wells (100 µL for flat-bottomed wells and 50 µL/well for round-bottomed wells). Unknown sera are diluted 200-fold and placed in adjacent wells. e.g. in rows A and B, starting at the top of column 2 of the tray. Column 1 is left empty until substrate is added before the final incubation step. It is used to set the blank for each optical channel before reading on the microtitre reader. The placing of replicate serum samples in adjacent optical channels minimises the effects of any errors in the blanks and makes such errors easily detectable by inspection of the readings. It also helps to detect edge-effects (i.e. anomalous behaviour of samples in the wells at the edges of the trays). Duplicate samples of seven serial two-fold dilutions of standard reference serum, or of a working reference serum calibrated against the standard, (starting with a 2000-fold dilution of the neat NSW reference serum) and of diluent alone, are placed in columns 6 and 7 of the tray. Further unknown samples are placed in columns 8–11, and column 12 receives PBST only. If the test is to be read by eye and the only information sought is whether or not the test sera exceed a predetermined level of reaction, then the inclusion of standards diluted to that level are all that is necessary. If the trays are to be read by spectrophotometer and a curve fitting program is being used to give the maximum precision, then standards at six or more different levels may be needed.

(d) Trays are covered and kept at room temperature for 1.5 hours. If a more rapid test is needed, can incubate for 45 min at 37°C in a fan-forced incubator with constant mechanical shaking of the trays. An incubator with fan-driven air circulation is advisable to ensure a constant and uniform temperature for all trays. Trays should not be stacked more than two high during incubation.

(e) Serum is shaken out and the trays are drained and washed four times as in (b).

(f) A suitable dilution of enzyme-antiglobulin conjugate in PBST is added to each well (100 µL for flat-bottomed wells and 50 µL/well for round-bottomed wells). Trays are covered and incubated at room temperature for 1.5 hours.

(g) Conjugate is shaken out and trays are washed four times and drained as in (b).

(h) Substrate solution (100 µL) is added to each well and trays are covered and shaken at room temperature for 30–60 min depending on the level of sensitivity required. The sensitivity of the test should be such that the cut-off point for diagnosis falls at least 0.2 absorbency units above the baseline.

(i) Results are assessed visually or in a spectrophotometer, before or after the addition of stopping reagents.

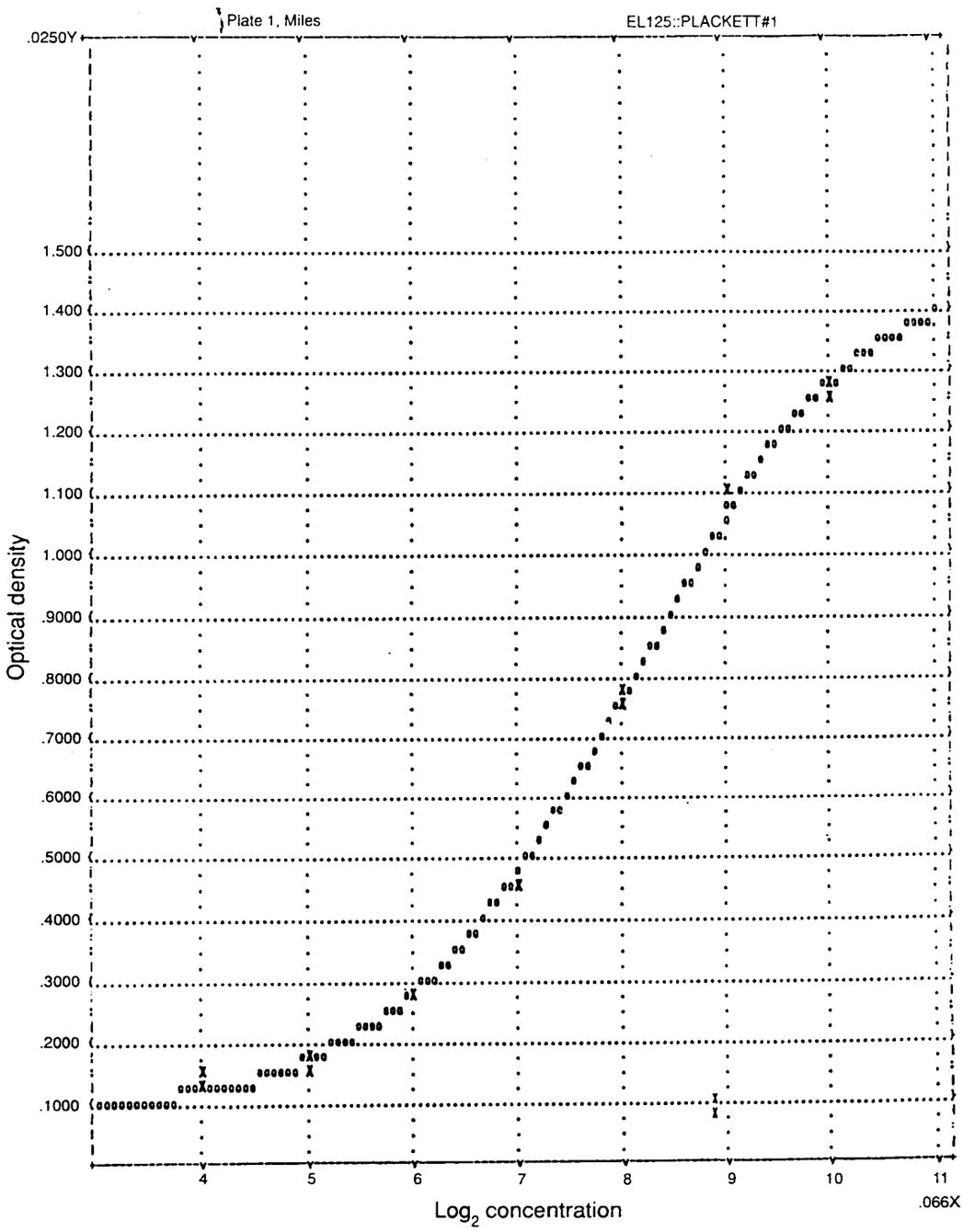


**Figure 3.** Logistic curve fitted to absorbance readings for dilutions of the New South Wales and anti-*Brucella abortus* reference sera. Ordinate: Absorbance at 450 nm. Abscissa: $Log_2$ (concentration of antibody units in serum dilution).

### 6.4. Quality Control and Data Processing

It is important to check the reproducibility of results both for variation between duplicates on each tray and for variations in test sensitivity between trays. This may be done by specifying limits within which absorbance readings given by appropriate dilutions of reference sera should lie. Tests on plates giving values outside these limits can then be repeated until satisfactory results are obtained. Detailed criteria have been developed for use in NSW State Laboratories (Searson, 1985). To allow standardisation between laboratories, results should be expressed in terms of the dilution of the standard serum giving the same absorbance as the test serum under the conditions used. The relationship between the dilution and the level of antibody in 'N units'/mL as defined above is:

N units/mL test serum =
(dilution of test serum)/(equivalent dilution of NSW standard serum) x 1024

It is particularly important to check sensitivity and reproducibility in the region of the cut off point: i.e., a 32 000-fold dilution of the reference serum, 64 N units/mL or 6 $\log_2$ units/mL.

It is recommended that the results be expressed on a logarithmic scale. If logs to base 2 are used, the results are related in a simple way to the steps in the two-fold dilution (dln) series used to obtain the data for the standard curve. Thus:

$\text{Log}_2$ (units/mL test serum)
= 10 + $\log_2$ (dln of test serum x10/equivalent dln of NSW reference serum).

Table 7 shows values covering the range of interest for tests on unknown sera diluted 200-fold .

The end point is 6 $\log_2$ units/mL. This value, based on extensive studies carried out in New South Wales, is applicable to unvaccinated cattle, the cut-off point for animals known to have been vaccinated with strain 19 being 7 $\log_2$ units/mL (J.E. Searson, pers. comm. 1992).

A plot of absorbance (OD) against the log of antibody concentration for the standard serum dilutions gives a sigmoid curve. The most appropriate equation for curve fitting is that of the four-parameter logistic function (Ukraincik and Piknosh, 1981). Suitable curve fitting programs are well within the capacity of the average microcomputer. A Fortran program for the purpose has been written by Leo Wursthorn of CSIRO Animal Health Laboratory, Parkville. It calculates the curve of best fit for the data obtained from the standard serum dilutions. Fig. 3 shows such a curve for a two-fold dilution series from 2000 to 128 000-fold, together with the observed duplicate OD values for each dilution (X,X). The program then calculates values for unknown samples in the same units, together with estimates of the standard deviation of the assay, and allows corrections to be made for variations in sensitivity due, e.g., to differences in incubation time or temperature. Details are available on request. A number of other programs are available, including some which do not assume a particular form of equation relating optical density to antibody units.

The absorbance values obtained in ELISA tests depend both on antibody concentration and affinity (Lehtonen and Eerola, 1982; Steward and Lew, 1985). The relationship between these quantities are complex, and the distribution of antibody affinities in polyclonal sera may be skewed or even bimodal (Steward and Steensgard, 1983). The ELISA readings given by high and low affinity antibodies are affected differently by changes in serum dilution and in the density of binding sites on microtitre wells. The objective is to establish standard conditions under which the test gives reproducible and consistent 'ELISA values' which can be used as a measure of the probability that the sample came from an infected animal.

**Table 7.** Calculation of $\text{Log}_2$ units for test serum diluted 1 in 200

| *Dilution of reference serum (x $10^{-3}$)* | *N units per mL of dilution* | *$\text{Log}_2$ units per mL test serum* |
|---|---|---|
| 2 | 1024 | 10 |
| 4 | 512 | 9 |
| 8 | 256 | 8 |
| 16 | 128 | 7 |
| 32 | 64 | 6 |
| 64 | 32 | 5 |
| 128 | 16 | 4 |
| 256 | 8 | 3 |

## 7. References


Anonymous (1965). Public Health Monograph No. 74. (United States Department of Health, Education and Welfare: Washington, DC.)

Alton, G.G., Jones, L.M., Angus, R.D., and Verger, J.M. (1988). 'Techniques in the Brucellosis Laboratory.' (Institut National de la Recherche Agronomique: Paris.)

Berman, D., Wilson, B., Moreno, E., Angus, R., and Jones, L. (1980). Characterization of *Brucella abortus* soluble antigen employed in immunoassay. *Journal of Clinical Microbiology* **11**, 355–62.

Campbell, D.H., Garvey, J.S., Cremer, N.E., and Sussdorf, D.H. (1970). 'Methods in Immunology: A Laboratory Text for Instruction and Research.' (Benjamin: Reading, Mass.,USA.)

Cruickshank, R., Duguid, J.P., Marion, B.P., and Swain, R.H.A. (1973). 'Medical Microbiology.' (Churchill Livingstone: London.)

Ellens, D.J., and Gielkens, A.L.J. (1980). A simple method for the purification of 5-aminosalicylic acid. Application of the product as a substrate in enzyme-linked immuno-sorbent assay (ELISA). *Journal of Immunological Methods* **37**, 325–32.

FAO/WHO (1971). The Joint FAO/WHO Expert Committee on Brucellosis 5th Report, WHO Technical Report Series No. 464. (World Health Organisation: Geneva.)

Kabat, E.A., and Mayer, M.M. (1961). 'Experimental Immunochemistry.' 2rd Edn. (Charles C. Thomas: Springfield, Ill., USA.)

Lehtonen, O.-P. and Eerola, E. (1982). The effect of different antibody affinities on ELISA absorbance and titer. *Journal of Immunological Methods* **54**, 233–40.

Sax, N.I. (1984). 'Dangerous Properties of Industrial Materials'. 6th Edn. (Van Nostrand Reinhold: New York.)

Searson, J.E. (1985). 'SCA Workshop on BTEC in Settled Areas of Australia.' (New South Wales Department of Agriculture: Sydney.)

Stewart, M.W., and Lew, A.M. (1985). The importance of antibody affinity in the performance of immunoassays for antibody. *Journal of Immunological Methods* **78**, 173–90.

Steward, M.W., and Steensgard, J. (1983). 'Antibody Affinity: Thermodynamic Aspects and Biological Significance.' p. 79. (CRC Press: Boca Raton, USA.)

Ukraincik, K., and Piknosh, W. (1981). Microprocessor based radioimmunoassay data analysis. *Methods in Enzymology* **74**, 497–508.

## 8. Appendixes

### *8.1. Appendix 1 — Kolmer Diluent*

#### 8.1.1. Stock Solution

| | |
|---|---|
| Calcium chloride, $CaCl_2.2H_2O$ | 24.5 g |
| Magnesium chloride hexahydrate, $MgCl_2.6H_2O$ | 101.6 g |
| Distilled water to | 500 mL |

Store in refrigerator.

#### 8.1.2. Preparation of Diluent

| | |
|---|---|
| Sodium chloride, NaCl | 17 g |
| Stock calcium and magnesium solution (see 8.1.1.) | 1.25 mL |
| Sodium azide, $NaN_3$ (10% w/v, 1.5 mol/L) | 0.25 mL |
| Distilled water to | 2 L |

Store at room temperature.

### *8.2. Appendix 2 — Alsever's Solution*

| | |
|---|---|
| Glucose, $C_6H_6O_6$ | 20.5 g |
| Sodium chloride | 4.2 g |
| Trisodium citrate, $Na_3C_6H_7.2H_2O$ | 8.0 g |
| Citric acid, $C_6H_8O_7$ | 0.6 g |
| Distilled (preferably) or deionised water | 1 L |

Check pH is 6.1.
Dissolve without heating.
Sterilise by filtration or in the autoclave at 115°C for 20 min.

### *8.3. Appendix 3 — Calibration of Spectrophotometer using Cyanmethaemoglobin Standard*

A 3% suspension of sheep erythrocytes prepared as described in 3.2.4. should contain 0.95 g haemoglobin (Hb) per 100 mL. This fact can be utilised in standardising erythrocyte suspensions photometrically. The spectrophotometer can be calibrated to indicate a given concentration of Hb using the cyanmethaemoglobin method, then for routine use this calibration can be converted to the equivalent OD of an erythrocyte suspension lysed in distilled water.

(a) Switch on spectrophotometer, set wavelength at 541 nm, or select the appropriate filter, allow to warm up.
(b) Bring cyanmethaemoglobin standard to room temperature.
(c) Read the OD of the standard on the spectrophotometer, using Drabkin's reagent as blank. (We recommend that Aculute diluent pellets be used for making up the Drabkin's reagent — available from Ortho Diagnostic Division of Ethnor Pty. Ltd, 1 Khartoum Road, North Ryde, NSW, Australia.)
(d) Calculate the OD for a suspension of sheep erythrocytes containing 0.95 g Hb per 100 mL, diluted 1:16 in Drabkin's reagent from the following formula:

OD = [(0.95 x OD of standard x 1000)]/ [Conc. of Standard (mg per 100 mL) x 16]

(e) Prepare a suspension of sheep erythrocytes about (4%).
(f) Mix 1 mL of this suspension with 15 mL Drabkin's reagent, allow to stand for 10 min, then determine the OD.
(g) Calculate the dilution factor required to bring the sheep erythrocyte suspension to the correct concentration (0.95 g haemoglobin per 100 mL) using the formula:

mL to be added =
(OD obtained–OD required)
x No. of mL to be diluted

(h) Make the necessary dilution and check that this is correct by mixing 1 mL with 15 mL Drabkin's reagent and reading the OD.
(i) Determine the target OD for routine use by lysing 1 mL of the sheep erythrocyte suspension prepared in (h) above in 15 mL distilled water and reading the OD in the spectrophotometer.

### *8.4. Appendix 4 —Percentage Haemolysis and [y/(100 - y)] for Spectrophotometer with Target OD = 0.5*

See Table 7.

### *8.5. Appendix 5 — Preparation of Crude Lipopolysaccharide from* Brucella abortus

ELISA LPS *Brucella abortus* antigen is available from: Dr J.E. Searson, Regional Veterinary Laboratory, Department of Agriculture, Wagga Wagga, NSW 2650, Australia.

*B. abortus*, strain 19, is grown on a modified glycerol–dextrose-agar medium (GDA-M, see 8.6.) in 80 Roux flasks of 1 L capacity. After four days at 37°C 25 mL of 0.5% (w/v) phenol in distilled water (4°C) is added to each flask. The growth is

Table 7. Percentage haemolysis and $y/(100-y)$ for spectrophotometer with target OD=0.5

| *Spectro reading* | *Per cent Haemolysis* | *y/100-y* | *Spectro reading* | *Per cent Haemolysis* | *y/100-y* |
|---|---|---|---|---|---|
| 0.45 | 90 | 9.0 | 0.255 | 51 | 1.04 |
| 0.445 | 89 | 8.09 | 0.25 | 50 | 1.0 |
| 0.44 | 88 | 7.33 | 0.245 | 49 | 0.96 |
| 0.435 | 87 | 6.69 | 0.24 | 48 | 0.92 |
| 0.43 | 86 | 6.14 | 0.235 | 47 | 0.89 |
| 0.425 | 85 | 5.67 | 0.23 | 46 | 0.85 |
| 0.42 | 84 | 5.25 | 0.225 | 45 | 0.82 |
| 0.415 | 83 | 4.88 | 0.22 | 44 | 0.79 |
| 0.41 | 82 | 4.56 | 0.215 | 43 | 0.75 |
| 0.405 | 81 | 4.26 | 0.21 | 42 | 0.72 |
| 0.4 | 80 | 4.0 | 0.205 | 41 | 0.69 |
| 0.395 | 79 | 3.76 | 0.2 | 40 | 0.67 |
| 0.39 | 78 | 3.55 | 0.195 | 39 | 0.64 |
| 0.385 | 77 | 3.35 | 0.19 | 38 | 0.61 |
| 0.38 | 76 | 3.17 | 0.185 | 37 | 0.59 |
| 0.375 | 75 | 3.0 | 0.18 | 36 | 0.56 |
| 0.37 | 74 | 2.85 | 0.175 | 35 | 0.54 |
| 0.365 | 73 | 2.70 | 0.17 | 34 | 0.52 |
| 0.36 | 72 | 2.57 | 0.165 | 33 | 0.49 |
| 0.355 | 71 | 2.45 | 0.16 | 32 | 0.47 |
| 0.35 | 70 | 2.33 | 0.155 | 31 | 0.45 |
| 0.345 | 69 | 2.23 | 0.15 | 30 | 0.43 |
| 0.34 | 68 | 2.13 | 0.145 | 29 | 0.41 |
| 0.335 | 67 | 2.03 | 0.14 | 28 | 0.39 |
| 0.33 | 66 | 1.94 | 0.135 | 27 | 0.37 |
| 0.325 | 65 | 1.86 | 0.13 | 26 | 0.35 |
| 0.32 | 64 | 1.78 | 0.125 | 25 | 0.33 |
| 0.315 | 63 | 1.70 | 0.12 | 24 | 0.32 |
| 0.31 | 62 | 1.63 | 0.115 | 23 | 0.30 |
| 0.305 | 61 | 1.56 | 0.11 | 22 | 0.28 |
| 0.3 | 60 | 1.5 | 0.105 | 21 | 0.27 |
| 0.295 | 59 | 1.44 | 0.1 | 20 | 0.25 |
| 0.29 | 58 | 1.38 | 0.095 | 19 | 0.23 |
| 0.285 | 57 | 1.33 | 0.09 | 18 | 0.22 |
| 0.28 | 56 | 1.27 | 0.185 | 17 | 0.20 |
| 0.275 | 55 | 1.22 | 0.18 | 16 | 0.19 |
| 0.27 | 54 | 1.17 | 0.175 | 15 | 0.18 |
| 0.265 | 53 | 1.13 | 0.17 | 14 | 0.16 |
| 0.26 | 52 | 1.08 | 0.165 | 13 | 0.15 |

suspended by gentle transverse shaking. The suspension is filtered through several layers of gauze into tared centrifuge bottles and the cells are resuspended in about 1.6 L of cold 0.5% phenol and centrifuged again. The pellets are drained and weighed. Eighty Roux flasks should yield about 200 g wet weight of packed cells.

The pellets are resuspended in distilled water (400 mL per 100 g of packed wet cells) and autoclaved for 20 min at 121°C (Berman *et al.*, 1980). After centrifugation the supernatant is separated and clarified by recentrifugation and/or by passage through a 0.45 µm Millipore filter. It is then made to 0.25 mol/L in sodium hydroxide by adding 1/15 of its volume of 4 mol/L sodium hydroxide, and is heated at 56°C for one hour. It is important that the period of heating is measured from the time the solution reaches 56°C. The solution is cooled, neutralised with glacial acetic acid ($CH_3CO_2H$) to pH 7.0, and mixed with four volumes of ethanol ($C_2H_5OH$). After standing at 4°C for 24–48 hours, the flocculent precipitate is collected by centrifugation or filtration (e.g. on a Whatman No. 50 filter paper). It is taken up in a small volume of distilled water and dialysed to remove salts and residual ethanol. The sac contents are centrifuged if necessary to remove any insoluble material. Samples are freeze dried in tared vials to determine the concentration. The stock solution may be stored frozen. Freeze-dried material may be kept in sealed ampules or in a desiccator at room temperature.

### *8.6. Appendix 6 — Modified Glycerol Dextrose Agar (GDA-M)*

| | |
|---|---|
| Bacto peptone | 10 g |
| Lab Lemco powder | 5 g |
| Sodium chloride | 5 g |
| Bacto agar | 25 g |
| Distilled water | 100 g |
| pH of basal medium | 7.0 |

Heat to dissolve agar then add (to give final concentration):

| | |
|---|---|
| Dextrose, $C_6H_{12}O_6$ | 0.1% |
| Glycerol, $C_3H_8O_3$ | 2.0% |
| Sodium metabisulfite, $Na_2S_2O_5$ | 0.01% |
| Bacto yeast extract | 0.2% |
| Final pH | 7.0 |

Dispense into Roux flasks and autoclave at 120°C for 30 min.

# ATTACHMENT VI

# ANIMAL HEALTH SURVEILLANCE QUARTERLY

*Newsletter of Australia's National Animal Health Information System*

**Volume 3** **Issue 1**

**Quarterly Report for 1 January to 31 March 1998**


## Preface

Ovine Johne's disease is receiving much attention this year, and this issue of *Animal Health Surveillance Quarterly* summarises activity to date. It also gives details of the findings of a recent review of Australia's animal health system. In addition, there are items of interest from States and Territories.

This newsletter summarises the findings of disease surveillance and monitoring activities reported to the National Animal Health Information System (NAHIS) for the period from 1 January to 31 March 1998. Only summary information is recorded in NAHIS, with detailed data being maintained by the source organisation. The information included in this report is accurate at the time of publication but, because of the short reporting and production time, minor discrepancies may occur.

I am sure that you will find this report to be useful.

GARDNER MURRAY
Australian Chief Veterinary Officer

## Ovine Johne's Disease — the National perspective

Johne's disease (JD) is a chronic enteric wasting disease, caused by *Mycobacterium paratuberculosis*. One form of the disease, ovine JD (OJD) was initially diagnosed in central New South Wales (NSW) in 1980, and for more than 10 years it was believed to be restricted to this area. More recently, it has become clear that OJD is present in southern areas of NSW. It has also been diagnosed in Victoria, on Flinders Island and in South Australia.

The need for a nationally coordinated JD control program, that includes OJD, has been recognised by both governments and industry. Although endemic disease control is a State responsibility, it was appreciated that nationally agreed standards and objectives were likely to allow State programs to result in the most efficient outcomes. In July 1995, the National Farmers' Federation appointed the National JD Coordinating Committee (NJDCC), to coordinate industry and government efforts. The NJDCC comprised representatives from the dairy, beef, sheep, goat, deer and alpaca industries as well as from the veterinary profession, research and development corporations, and governments. It aimed to reduce the national impact of JD in all species and to promote and assist industry's management of the disease, and facilitated considerable consultation with livestock industries and governments in 1996. In early 1997, the Australian Animal Health Council (AAHC) introduced the National JD Industry Liaison Committee (NJDILC), which took over from the NFF committee. A National JD Coordinator was appointed.

### Recent developments

As the wider distribution of OJD became apparent, the need for its control on a national basis became more evident and urgent (see Table 1). As a result of a nationally coordinated effort, two developments resulted — the launching of the Australian Sheep JD Market Assurance Program (MAP) in June 1997,

## Contents

**Table 1: Detection and destocking of infected flocks at 31 March 1998**

| State | No. of infected flocks detected | No. of infected Flocks destocked |
|---|---|---|
| NSW | 243 | 15 |
| VIC | 75 | 67 |
| TAS | 8 | 1 |
| SA | 1 | 1 |
| WA | 0 | 0 |
| QLD | 0 | 0 |
| NT | 0 | 0 |

and agreement on Standard Definitions and Rules for regulatory disease control in March 1998.

At the same time, calls were being made for a national OJD eradication program. Victoria had initiated a destocking program in late December 1996, with compensation being made available from State Government and industry funds. In May 1997, AAHC endorsed the NJDILC's recommendation that compulsory control and/or eradication programs in infected zones or at a national level should not be implemented unless, among other things, studies on economic cost–benefits and the distribution and prevalence of infection were completed.

During the following two months, the results of an economic analysis by the Australian Bureau of Agricultural and Resource Economics, and of surveillance for OJD in NSW , led to strong support within the sheep industry for a national program to eradicate OJD on a progressive and voluntary basis. This was supported in principle by NJDILC in July, subject to adequate financial assistance from government and livestock industries. Following the NJDILC recommendation, AAHC recommended that Commonwealth and State Ministers for Agriculture (ARMCANZ) endorse a Progressive Control and Eradication Program for OJD, with an indicative budget of $38.2 m over five years. This was endorsed 'subject to consultation within jurisdictions', and AAHC was requested to negotiate funding arrangements and conduct a thorough assessment of costs, for consideration out-of-session in October 1997.

Considerable activity occurred over the ensuing three months. Revised costings of the proposed program, proposals for financial assistance, and cost-sharing arrangements for funding the program were developed. The purpose of the proposed 'voluntary' program was to encourage, but not force, producers to participate in the program and undertake eradication by destocking in the initial stages. This was in recognition of concerns over the uncertainty of distribution of the disease (and therefore, in the ability to complete a national eradication program), as well as reservations over some technical aspects of eradication. This initial 'voluntary' stage would only progress to compulsory eradication if all concerns were adequately addressed.

The issue of funding the program was not resolved by October. Industry had made a commitment to fund up to 50% of the costs, but no agreement was reached on how the remaining 50% of the program would be funded. As a result of the unresolved funding issue, and increasing concern on the reliability of surveillance data, the AAHC Board recommended to ARMCANZ that further surveillance data to ascertain the feasibility of eradication, and further negotiations to determine funding arrangements, were required before a National Eradication Program could commence. An AAHC Technical Working Group on 'enhanced surveillance' was formed and reported to AAHC in early January.

In December, the Commonwealth Minister for Primary Industries commissioned Mr Denis Hussey and Professor Roger Morris to investigate the role that the Commonwealth might play in regard to OJD. This report, released in January 1998, recommended two three-year programs that maintained surveillance and control of the disease while evaluating tools and options for control and possible eradication, should that be agreed to at some future time. This approach was accepted by the livestock industries and by governments as the basis for dealing with OJD in future.

At its February meeting, ARMCANZ considered both the Hussey–Morris Report and the Enhanced Surveillance Report, and supported an immediate Interim Surveillance Monitoring and Research Program, on a 20% Commonwealth Government: 30% State Governments basis subject to industry commitment to fund the other 50%. It also requested a detailed Business Plan for the Evaluation Program to be developed for consideration in July.

As a result, AAHC announced a $2.45m Interim Surveillance and Research Program to be carried out from April 1 to July 31 1998. The key elements to the Interim Program are:

- tracing and investigations of 'at risk' flocks to support zoning declarations;
- MAP subsidies;
- evaluation of abattoir monitoring as a surveillance tool; and
- evaluation of pooled faecal culture test.

In addition, AAHC established a National OJD Committee to develop a detailed Business Plan for a six-year National OJD Control and Evaluation Program. Four technical working groups have been established to assist with this. It is expected that the Business Plan will be considered by the AAHC Board in early July, and submitted to ARMCANZ for consideration at the end of July. The Hussey–Morris Report will act as a basis for much of the Business Plan. Key elements to the six-year program are likely to be:

- evaluation of on-farm control and eradication strategies;
- completion of zoning testing and proclamations;
- increased surveillance to provide a high level of certainty of the distribution and prevalence of OJD, including abattoir monitoring and implementation of a sheep identification system; and
- increased research into improved tests.

AAHC has also indicated it will independently review the flow of benefits from any eradication program to further develop the appropriate funding arrangements for the six-year program, and any subsequent control/eradication program.

## Conclusion

The JD Program is a joint industry and government program, and there has been strong agreement that the approach to JD should be a national one. There has been considerable industry involvement and commitment to ensure the Program commenced, and moves for a National OJD Program have had strong industry support.

Despite this industry commitment at the national level, there has been concern, both within industry and from governments, over the accuracy of the relatively limited data on the prevalence and distribution of OJD, and the feasibility and cost-effectiveness of national eradication. As a result, getting agreement to fund a national OJD program has been difficult. Although there is general agreement from all parties of the extent of current knowledge and information, there remains disagreement on which course of action should be followed.

The long-term future of OJD control in Australia will depend heavily on the results of surveillance and evaluation projects in the next six years. If the disease is more widespread than currently estimated, or eradication at the farm level proves more difficult, the economic benefit of (and even the success of) large-scale regional programs would be questionable. However, if surveillance increases confidence that the disease has a limited distribution, and evaluation of property programs demonstrates that eradication is feasible, then a future national eradication program should be beneficial. If OJD is not eradicated, it will continue to spread, infecting increasing numbers of flocks over wider areas. Changes in management and vaccination of infected flocks may be widely implemented to limit the effects of the disease. Movement of sheep will probably be disrupted as protected and free zones attempt to keep the disease out and accept sheep only from MAP assessed flocks.

*Contributed by: MB Allworth and DJ Kennedy, National Coordinators, National Johne's Disease Program*

**Disease Watch Hotline – 1800 675 888**

The Disease Watch Hotline is a toll-free telephone number that connects callers to the relevant State or Territory officer to report concerns about any potential exotic disease situation. Anyone suspecting an exotic disease outbreak should use this number to get immediate advice and assistance.

*Contact: Chris Bunn, Emergency Disease Strategies Section (formerlyAnimal Diseases/Incidents), DPIE.*

Volume 3 Issue 1
Quarterly Report for 1 January to 31 March 1998

# Tuberculosis Freedom Assurance Program

The Tuberculosis Freedom Assurance Program (TFAP) commenced on 1 January 1998. This is a surveillance program to ensure that any resurgence of tuberculosis (TB) in Australian cattle is promptly and effectively eliminated. It follows the Brucellosis and Tuberculosis Eradication Campaign (BTEC) that successfully concluded on 31 December 1997 with the declaration of Australia as a Free Area for bovine tuberculosis (*AHSQ*, Vol. 2, No. 4).

Like BTEC, TFAP is a cooperative partnership between government and industry and each has an agreed role and funding obligation. In a major organisational change, AAHC coordinates funding and program management for the program. It employs a part-time National Coordinator who works with a Coordination Committee on which all stakeholders are represented.

The program is implemented by the States and Territories in accordance with a formal agreement between all of the parties involved and TFAP Standard Definitions and Rules. This agreement binds AAHC, the Commonwealth Government, the State and Northern Territory (NT) governments, and the Cattle Council of Australia to a set of operating principles that are broadly based on those that operated during BTEC. However, TFAP has a different set of funding arrangements to BTEC, with the different components being funding by specific parties, as outlined below.

## National Granuloma Submission Program

The National Granuloma Submission Program is the main TB surveillance process. It is funded by the Commonwealth Government and involves the Australian Quarantine and Inspection Service and States and Territories working together to maximise the number of granulomatous lesions identified in cattle at slaughter. Lesions are submitted to an approved laboratory and examined intensively, according to a national protocol, for evidence of TB.

## Field operations

These comprise all the activities necessary to effectively monitor for, diagnose and manage the eradication of tuberculosis in a particular State or the NT. This includes providing policy advice, legislation and all infrastructure to support diagnostic and eradication activities, including maintaining tracing systems, developing property programs, supervising test and slaughter programs and record maintenance. Field operations are funded and carried out by the States and NT according to the TFAP Agreement and the Standard Definitions and Rules.

## Australian Reference Laboratory

Funding for the Australian Reference Laboratory for Bovine Tuberculosis, an OIE-recognised World Reference Laboratory for TB, is provided by the Commonwealth Government. It is located within the WA Department of Agriculture at South Perth and is required to offer specialist diagnostic services, stay abreast of scientific developments in the field, and maintain an international profile.

## Tuberculosis Case Register

A TB Case Register is also funded by the Commonwealth Government. The register is managed by Queensland Department of Primary Industries and contains records of cases of TB found in Australia.

## Producer assistance measures

Producer assistance measures are funded by the Australian cattle industry. They include compensation to producers for reactor cattle and cattle destroyed as a result of control operations, support to muster and hold cattle to restock properties, and a subsidy on the costs of returning cattle to a property that has been depopulated as part of eradication procedures. An interest subsidy is available on loans to erect any additional infra-structure required to comply with the program's needs.

TFAP will continue until the 31 December 2002. A review in three years time will determine whether any program should follow its completion.

*Inquiries about TFAP should be directed to the TFAP Coordinator, Dr Geoff Neumann, c/- Australian Animal Health Council Limited, Suite 15, 26–28 Napier Close, Deakin ACT 2600*

Volume 3 Issue 1
Quarterly Report for 1 January to 31 March 1998

# Strategic priorities for animal health

A major review of Australia's animal health system was undertaken in 1997 to define strategic priorities for the next five years. A plan, covering 1998–2003, has been developed to deliver defined core animal health activities.

Under Australia's federal system, animal health services are provided under a number of separate and autonomous jurisdictions. It must be recognised that common national objectives will be managed and delivered on a regional basis, with varying approaches. The aim must be for consistency, rather than uniformity, to achieve national outcomes.

To meet its national and international obligations, Australia's animal health services need to meet certain minimum performance standards. These standards need to be determined and agreed, and subjected to ongoing monitoring that will give early warning of deficiencies and attract remedial action. This will not be an easy task and it is recommended that the laboratory sector be reviewed first, and that approaches be developed for reviewing other elements of the animal health system.

Stategies and actions proposed to achieve the shared vision for Australia's animal health include: legislative and administrative matters; involvement of the private sector; surveillance, diagnosis and reporting; animal welfare; food safety and residues; and emergency preparedness.

Some of these actions are already underway, and most are expected to start within the next 12 months; all should be completed within five years. Undoubtedly, other challenges and opportunities will also occur during this period.

The immediate outcomes required are:

- commitment to the principles outlined in the strategic priorities plan;
- endorsement of the roles and responsibilities of the key stakeholders, as a basis for negotiation on policy, service delivery and funding; and
- agreement to develop and monitor performance standards for Australia's animal health services.

A range of events such as chemical residues, toxigenic *E. coli*, and the occurrence of bovine spongiform encephalopathy oversease has sensitised the Australian and overseas public to food health issues. More emphasis on accountability at all stages of production of livestock and livestock products is now essential. The development and application of quality assurance schemes using HACCP principles will be an essential part of all future food production systems. This approach will be supported by a continuing effort at disease prevention within production systems

To achieve national outcomes efficiently, Australia's animal health service needs to have a clear focus in the broadest, collective sense. To achieve its purpose of protecting consumers, reducing the impact of disease on market access and production, and applying appropriate animal welfare standards, Australia's animal health services will have a number of core functions. These are to:

- protect domestic and other consumers from both real and perceived risks from residues, contaminants, and disease agents;
- enable continuous, competitive access to all export markets;
- provide harmonised, cost-effective and efficient service delivery arrangements throughout Australia;
- prevent entry and establishment of unwanted pests and pathogens;
- provide sufficient, well organised and succinctly presented information on Australia's animal health status to meet international, national and sub-national requirements;
- minimise the impact on production and trade of animal diseases;
- manage emergency and exotic disease and residue incidences in a cost effective manner with minimal disruption to trade; and
- meet the animal welfare standards of the community and trading partners.

*Peter Thornber*
*Office of the Chief Veterinary Officer*

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# State and Territory Reports

## New South Wales

*Contributed by:*
*Evan Sergeant*
*NSW Agriculture*

### Anthrax

There were nine anthrax incidents diagnosed during the quarter. A group of four cases occurred in the central-west area, on adjoining properties and adjoining paddocks — two in Molong Rural Lands Protection Board (RLPB) and two in Dubbo RLPB. One property had cattle deaths last year for which a diagnosis was never confirmed and that may have been due to anthrax. This may have contributed to a build-up of soil contamination leading to this year's cases. Another pair of almost adjacent properties in Narrandera RLPB had cases. Again, one property had a history of anthrax 40 years ago. All these cases occurred in areas that have a history of anthrax in the early 1940s but have not had any recognised cases in recent years.

The remaining cases involved pigs and sheep on two adjoining properties in Hay RLPB, and cattle on a property in the Murray RLPB.

This brings the total number of incidents for the 1997–98 summer to 13 — well within normal expectations, considering the hot, dry conditions experienced. Laboratory examination excluded anthrax as the cause of death in 10 other disease investigations during the quarter.

### Cattle tick control program

Fifty-three properties infested with cattle tick have been detected up to 31 March 1998. Four of these were properties detected in 1997 where mustering problems had permitted infestations to persist. Fourteen properties adjoined infested properties in 1997.

Thirty-one are within the Cattle Tick Protected Area. The remaining 22 are within the former Tick Quarantine Area or former Cattle Tick Protected Area (1996–97). Detailed chemical resistance testing and DNA testing will be done. However, it is already clear that some of the infestations detected in 1998 relate directly to 1997 infestations and have been spread through unreported livestock movements.

### Tick fever in NSW

A single case of tick fever was reported from a property near Urbenville. *Babesia bovis* was diagnosed at post mortem examination. No further cases were observed, and cattle ticks have not been detected on the property. Serological examination of the herd provided equivocal evidence of infection, with some sero-positive animals being sero-negative within two to three weeks. As a precaution, at-risk animals were treated and a cattle tick eradication program is being carried out on the property.

### Enzootic bovine leucosis

The 16th round of bulk milk testing (BMT) for enzootic bovine leucosis (EBL) was completed in March, with only 4.7% of the 1796 dairies being positive. At the beginning of the EBL Eradication Program in 1992, more than 25% of tested dairy herds produced positive BMT results. There are still 209 dairy herds in NSW classified as 'infected'. However, the testing indicates that the estimated within-herd prevalence is less than 2% in some 59% of the infected herds, suggesting that more than half of these infected herds may have removed all known EBL reactors for slaughter.

In the March 98 BMT test round, six dairy herds produced an unexpected positive BMT result. The herds are under investigation and preliminary findings indicate that vat milk sampling errors or unintentional introduction of EBL-infected animals may have been responsible for the positive results.

### Bat viruses

During the quarter, the 26 fruit bats and two micro-bats examined for evidence of lyssavirus infection gave negative results. Only nine fruit bats have been found to be infected from the 150 fruit bats examined for lyssavirus, while all 34 micro-bats examined have been negative.

Six horses examined for evidence of bat paramyxo-virus infection during the quarter had negative

results. A total of 91 fruit bats, 12 micro-bats and 11 horses have been examined for evidence of paramyxovirus infection, with one fruit bat serologically positive and one virologically positive.

### Avian tuberculosis

Avian tuberculosis caused the deaths of four 10-month-old pullets from Yass. Pathologically, there was multifocal granulomatous hepatitis and splenitis, and *Mycobacterium avium* complex was recovered from the affected organs.

### Toxoplasmosis survey

Results from a national pig industry survey of the prevalence of *Toxoplasma gondii* in feral pigs, commercial indoor and outdoor sows and finisher pigs, have been released by the Pig Research and Development Corporation.

The national serological prevalence of toxoplasmosis was estimated as 1.3% for finisher pigs, 11.5% for indoor sows, 6.7% for outside sows and 9.3% for feral pigs. NSW figures were comparable to other States' and compare favourably with those recorded in our major trading partners.

### Bee diseases

A further 139 tests for American foulbrood testing were done, bringing the total number of tests since July 1997 to 695. Almost exactly 50% of tests were positives. Of the 347 positive reports, 110 were repeat tests on previously confirmed cases. Of the 237 new cases, 78 also had positive reports during the 1996–97 financial year. Two reports of chalkbrood were received during the quarter.

### Exotic disease investigations

Following the recent avian influenza (AI) outbreak at Tamworth, increased awareness of exotic disease in poultry resulted in a number of cases being submitted for exclusion testing for AI.

Investigations were also carried out on high mortalities in a breeding complex at Tamworth to exclude AI, and on a suspect Newcastle disease outbreak in commercial layers in Sydney.

## Northern Territory

*Contributed by:*
*Diana Pinch*
*NT DPIF*

### Cattle

Urea poisoning caused deaths in cattle fed a high urea (25%) wet season mineral mix in the Darwin region. The deaths were associated with supplement sites that had previously run out of supplement, and no mortalities were observed at sites where the mix remained available at all times.

The Declared Tick Areas for the cattle tick *Boophilus microplus* have been modified following surveillance during 1997. The southern area of the NT is tick free, and the recent changes have extended this area northwards.

### Poultry

About 40 out of 80 backyard poultry (ducks and chickens) died over the course of a week in the Darwin region. Laboratory investigations indicated the deaths were caused by an outbreak of botulism due to type C toxin.

### Crocodiles

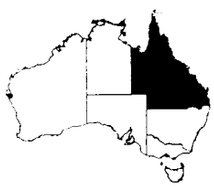
Increased mortalities in yearling crocodiles were reported at one farm. The animals became inappetant, developed 'fungal' skin lesions, lost condition and died. Septicaemia secondary to stress associated with recent changes in management was suspected. This was confirmed, with bacterial septicaemias due to several organisms that responded to antibiotic treatment and improved husbandry.

**NAHIS web site**

**http://www.brs.gov.au/aphb/aha**

This newsletter is available on the NAHIS website, which provides information and statistics about animal health matters in Australia.

## Queensland



*Contributed by:*
*Gavin Ramsay*
*Queensland DPI*

### TSE surveillance

Workshops have been held to provide Departmental staff with specialist skills in the areas of recognition of signs of neurological disease and the collection, preservation and submission of specimens for TSE exclusion. Specimens have been submitted to veterinary laboratories and results will be available for the next quarterly report.

### Tick fever

Cases of tick fever due to *Babesia. bovis*, *B. bigemina* and *Anaplasma marginale* have been reported in central, south and south-east Queensland. The variation in rainfall between years has been a contributing factor with increased tick populations in some areas this year. Producers are again being advised of the benefits of vaccination against these diseases.

### Bovine ephemeral fever

Bovine ephemeral fever has been reported from south-east and central regions, with northern Queensland expecting cases following high rainfall in recent months. Case severity has been less severe in the south-east but more severe in central region than usual. The west region has not reported any cases of bovine ephemeral fever this year.

### Internal parasites

Disease due to internal parasites has been diagnosed in young cattle in good condition on several properties in the north region following good rains. *Haemonchus* and *Cooperia* have been the main parasites each often accounting for almost 100% of faecal culture. Further sampling of older stock on one property has also demonstrated high parasite burdens.

### Sheep diseases

'Humpy back' was widely reported in sheep in the west region during February and March. Several sheep with suspected humpy back have been autopsied. Some were found to have Wallerian degeneration in the spinal cord ,as is generally seen in cases of humpy back. However, a ram from Winton, suspected of having humpy back, was diagnosed as suffering from a skeletal myopathy.

### Poisoning

*Georgina gidyea* was found in a paddock in western Queensland where unexplained deaths had been occurring in breeding cattle. Since trees vary in the amount of toxin produced, samples from the trees are being tested for fluoroacetate to confirm the diagnosis.

## South Australia

*Contributed by:*
*Kim Critchley*
*Primary Industries SA*

### Pyrrolizidine alkaloid concerns

Although there has been little rain of benefit to producers, some areas had heavy local falls just pre-harvest and, as a consequence, a dense germination of potato weed. Producers are now aware of the problems this weed can cause, and manage stock accordingly. Feed mills are also taking precautions and sample grain for potato weed seed. The only report of probable toxicity was in some calves being extensively managed.

### Vibrio in abalone

An organism similar to *Vibrio parahaemolyticum* was cultured from specimens obtained from a commercial abalone farm that had extensive mortality. Oxytetracycline medication added to the water appears to have resolved the problem.

### Mulberry heart disease

There is a quite high prevalence of mulberry heart disease in South Australia. Many of the State's small piggeries are home-mixers, and the nutrient content is often inconsistent and inadequate. The typical syndrome is sudden death in well grown pigs with confirmation of typical lesions of vitamin E/ selenium deficiency on post mortem examination.

### Monitoring program for boar semen

Concerns had been expressed at a suspected lowered fertility of chilled boar semen from one enterprise. Studies indicated there may have been problems with the antibiotics used to assist with bacterial control. Although it is only a short time since the antibiotics were changed, the results look promising.

### Johne's disease in alpaca

Last quarter there was a report of positive *Mycobacterium paratuberculosis* faecal culture in two alpaca from different properties. Traceback failed to identify a likely source, and histological and bacterial examination of the slaughtered animal was negative. The other animal is quarantined and, having returned a negative faecal test, will now be subjected to a number of sequential faecal culture tests.

## Tasmania

*Contributed by:*
*Rod Andrewartha*
*DPIF, Tasmania*

### Fruit bats on King Island

Although there are no fruit bats on King Island, one, presumably lost, was captured when it became tangled in netting over fruit trees on the island. Although there were no clinical signs of disease, fixed brain and blood samples collected from a flying fox submitted live from King Island were sent to the Australian Animal Health Laboratories (AAHL) for testing for lyssavirus and morbillivirues. The blood samples were positive for bat paramyxovirus on serology but brain samples were negative for both bat paramyxovirus and lyssavirus.

### Fish virus being investigated

An aquabirnavirus was detected in farmed finfish in Macquarie Harbour on the west coast of Tasmania. The virus, not seen before in the State, was detected as an incidental finding during routine fish health surveillance and was not associated with any significant outbreak of disease in the fish.

Surveillance testing is being carried out to determine the distribution of the virus and the species affected. Pathogenicity testing is under way at AAHL to determine the significance of the virus. Farmers in the area where the virus was detected are cooperating with the Department pending clarification of the significance of the finding. This involves a restriction on the movement of broodstock from the area.

### Hepatotoxic condition in cattle

Mortality of about 60% occured in a herd of Angus cattle very soon after it was placed onto an old oaten stubble pasture (with other old senescent grass) and some green regrowth coming through the dead litter. Histopathology of the liver was consistent with a condition recognised in Tasmania, generally in the spring or autumn, and believed to be caused by an unknown pasture toxin. The cause of death is massive destruction of liver cells ,and the condition can strike suddenly with animals dying within 12 hours of access to toxic pasture. As the cause cannot be recognised in the pasture, the only recommendation is to avoid letting pastures go rank and dry off. Heavy grazing with sheep to remove the excess pasture is recommended as sheep appear to be immune to the problem. Recovered animals show signs of photosensitisation. Other outbreaks in other years have resulted in losses of 50 to 70 cows in dairy herds.

## Victoria

*Contributed by:*
*John Galvin*
*Agriculture Victoria*

### Anthrax

In north-central Victoria, re-vaccination of cattle on properties where anthrax occurred during early 1997, and on adjoining properties, was completed by mid-November 1997. This involved vaccinating more than 50 000 cattle on 291 properties. From January to March 1998, one case of anthrax was confirmed. This was a vaccinated calf that died on a property on which multiple cases of anthrax occurred during 1997.

Surveillance for anthrax was established at a local knackery in January 1998, as was done during the 1997 outbreak. From January until the end of March,

more than 680 cattle were tested without any cases of anthrax discovered. These cattle originated from a wide area across north-central Victoria. The results indicate that the preventive program put in place after the 1997 outbreak has been a major success. All properties that vaccinated during 1997 will be required to vaccinate again during 1998 under a program that is jointly funded by industry, producers and the Victorian Government.

### Ovine Johne's disease

Since OJD was diagnosed in Victoria in November 1995, some 75 infected flocks have been detected. The first 33 flocks that were detected before the summer of 1996–97 were all destocked over that summer period. A further 33 flocks were detected by December 1997. At the end of March 1998, there are eight remaining infected flocks — all others have been destocked to eradicate the disease.

More than 600 farms have been investigated since November 1995, and about 1000 on-farm investigations conducted, with more than 20 000 sheep blood tested for OJD. The number of flocks detected as OJD-infected compared to the total number of farms investigated has decreased from 18.1% in January 1997 to 12.2% in March 1998. The level of active surveillance will increase under the national OJD program.

### Plant poisonings of stock

Millet hay was purchased for drought-affected beef cattle in East Gippsland and 11 died after feeding. Analysis of the hay showed very high nitrate levels and the deaths were attributed to nitrate toxicity. Lupinosis was reported as the cause of death of a number of sheep in central Victoria following the summer rains.

Caltrop (*Tribulus terrestris*) is a summer weed that has been associated with severe jaundice and death in sheep on several occasions in north-west Victoria. The nervous form of the disease recorded in the scientific literature has not been reported in Victoria. Caltrop poisoning was the cause of deaths in several mobs of lambs in the Victorian Mallee, where 50 of 400, and 140 of 350 lambs died with signs of acute liver failure.

## Western Australia

*Contributed by:*
*Richard Norris*
*Agriculture WA*

### Ovine Johne's disease

Western Australia is involved in two key activities as part of the nationally agreed Interim Surveillance and Research Program. The first activity provides information about the level of 'false positives' found by a proposed technique to detect OJD — adult sheep slaughtered at an abattoir are being examined for gross lesions of OJD in the gastrointestinal tract and regional lymph nodes. If any suspicious lesions are seen, samples are sent for histopathological examination.

The second activity involves blood testing of sheep from 'at risk' flocks — those that have imported sheep from New Zealand or certain areas of Australia. The testing is being done as part of the national program and not because there is a suspicion that infection is present. It will provide additional evidence of Western Australia's freedom from OJD

### Liver fluke

Health requirements for stock entering Western Australia are to be tightened to ensure that imported animals are free of liver fluke. This action follows the discovery of liver fluke in 29 imported horses in 1997. Targeted surveillance detected that liver fluke survived treatments given as part of the import protocol, and the fluke were resistant to repeated conventional treatment. Fortunately, follow-up surveillance has indicated that the fluke were eradicated following special treatment.

### TSE surveillance

Surveillance for transmissible spongiform encephalopathy (TSE) has commenced in Western Australia. Veterinarians have been asked to submit brain and other tissues from sheep or cattle with nervous signs, for TSE exclusion and differential diagnosis. During the quarter, brains from four sheep were examined histologically for evidence of TSEs, with negative results.

### Chalkbrood disease

Chalkbrood disease has recently been found in several apiaries in Western Australia. An emergency response campaign was undertaken following initial detection. However, surveillance showed that infection was established in several apiaries in the metropolitan and southern areas of the State.

### Veterinary Services technical update

A recent meeting of 24 Government Veterinary Officers located from Kununurra to Esperance provided an opportunity to update technical skills in collaboration with pathologists from the Department's Animal Health Laboratories. The meeting focused on recognition, diagnosis and sample submission for a range of exotic and endemic diseases in livestock and poultry.

Surveillance in the northern pastoral areas of the State will be enhanced with the recent appointment of an additional veterinarian at Kununurra and a new veterinary officer at Karratha. An essential part of their duties will be conducting disease investigations and establishing a surveillance network involving pastoralists and others in the region.

# Quarterly Disease Statistics

## Control activities

### Enzootic bovine leucosis

EBL accreditation programs have been operating in the dairy industries in Queensland and NSW for several years. Victoria, South Australia, Western Australia and Tasmania are undertaking a program of bulk milk testing of all dairy herds. Table 2 shows the number of dairy herds tested free at the end of the quarter.

**Table 2: Dairy herds tested free of EBL at 31 March 1998**

| | NSW | NT | QLD | SA | TAS | VIC | WA | AUST |
|---|---|---|---|---|---|---|---|---|
| Free | 1473 | 0 | 1735 | 762 | 719 | 6481 | 455 | 11 625 |
| Herds | 1795 | 0 | 2026 | 787 | 810 | 8453 | 467 | 14 338 |

### Ovine brucellosis

Accreditation programs for ovine brucellosis freedom are operating in most States. Table 3 shows the number of accredited flocks at the end of the quarter.

**Table 3: Ovine brucellosis accredited free flocks at 31 March 1998**

| NSW | NT | QLD | SA | TAS | VIC | WA | AUST |
|---|---|---|---|---|---|---|---|
| 1280 | 0 | 71 | 554 | 150 | 767 | 86 | 2908 |

### Tuberculosis

Australia was declared a Free Area for bovine tuberculosis on 31 December 1997. The National Granuloma Submission Program is the major surveillance method for TB under the Tuberculosis Freedom Assurance program. Table 4 summarises results from the Program. There were no cases of TB detected in the current quarter in the 508 granulomas that were submitted.

**Table 4: Results of the National Granuloma Submission Program**

| | Granulomas Submitted | TB +ve |
|---|---|---|
| Jan - Mar 97 | 1043 | 0 |
| Apr - Jun 97 | 943 | 1 |
| Jul - Sep 97 | 1464 | 4 |
| Oct - Dec 97 | 848 | 0 |
| Jan - Mar 98 | 508 | 0 |
| NSW | 79 | 0 |
| NT | 0 | 0 |
| QLD | 288 | 0 |
| SA | 17 | 0 |
| TAS | 50 | 0 |
| VIC | 4 | 0 |
| WA | 70 | 0 |

## Johne's disease

JD is seen primarily in dairy cattle but occurs occasionally in beef cattle, sheep and dairy goats, and has been diagnosed in a small number of alpacas. JD occurs in NSW, Victoria, Tasmania and South Australia. Surveillance programs support the view that cattle in Queensland, Western Australia and Northern Territory are free of JD, and active measures are taken to stamp-out any incursions. There are also Market Assurance Programs (MAP). Table 5 shows the number of herds and flocks known or suspected to be infected.

**Table 5: Herds/flocks with JD at 31 March 1998**

| | Cattle | Sheep | Goats | Alpacas | Total |
|---|---|---|---|---|---|
| NSW | 135 | 161 | 4 | 1 | 301 |
| NT | 0 | 0 | 0 | 0 | 0 |
| QLD | 0 | 0 | 0 | 0 | 0 |
| SA | 27 | 0 | 0 | 0 | 27 |
| TAS | 32 | 7 | 9 | 0 | 48 |
| VIC | 1643 | 42 | 0 | 11 | 1696 |
| WA | 0 | 0 | 0 | 0 | 0 |
| AUST | 1837 | 210 | 13 | 12 | 2072 |

## CattleMAP

The revised MAP for cattle, CattleMAP, was endorsed by Veterinary Committee and AAHC in March. Fifteen hundred copies of the Rules and Guidelines have been printed for distribution to owners of assessed herds, approved veterinarians and other interested people.

The major change in the CattleMAP is the consolidation of the Tested Negative (TN) and Monitored Negative (MN) testing streams into one MN stream. Herd status in the original National JDMAP is transferred to the CattleMAP. All herds at the same level (1, 2 or 3) have equivalent status and cattle can move freely between TN1 and MN1 herds and between TN2 and MN2 herds. TN herds will progress to the next highest MN status at their next negative test and assessment (i.e. from TN1 to MN2, TN2 to MN3). With this change, the testing now involves cattle over two years of age. In herds with up to 210 cattle over two years of age, all such cattle will be tested. In larger herds a sample of up to 300 cattle will be tested.

The other main change is that, under certain conditions, assessed herds can now source bulls from traditional bull supply herds that are one status lower.

The number of assessed herds in CattleMAP increased to 416 (278 beef herds and 138 dairy herds) at 30 April 30, 1998.

## SheepMAP

At the end of March, 52 sheep flocks had been assessed under the SheepMAP — 7 in South Australia, 9 in Victoria and 36 in NSW. Merino or poll merino flocks constituted three quarters of these assessed flocks. To encourage flock testing, the Interim Surveillance and Research Program for OJD includes a subsidy for flock owners enrolling in the SheepMAP between April and July 1998.

*Further information about the various JD Market Assurance Programs can be obtained from David Kennedy 02 6365 6016 or Bruce Allworth 02 6936 9233. Lists of assessed beef and dairy herds and sheep flocks are available on a fax-back service on 1902 940 579.*

## Bovine brucellosis

Although bovine brucellosis is now exotic to Australia, surveillance is maintained through abortion investigations and miscellaneous testing of cattle for export or other reasons. A total of 79 abortion investigations were performed during the reporting period — all with negative results for bovine brucellosis. The results of recent brucellosis surveillance are shown in Table 6.

**Table 6: Surveillance for bovine brucellosis**

| | Abortion Investigations | | Test for other reasons | |
|---|---|---|---|---|
| | Tests | +ve | Tests | +ve |
| Jan - Mar 97 | 122 | 0 | 2288 | 0 |
| Apr - Jun 97 | 142 | 0 | 4336 | 0 |
| Jul - Sep 97 | 196 | 0 | 3956 | 0 |
| Oct - Dec 97 | 169 | 0 | 2847 | 0 |
| Jan - Mar 98 | 79 | 0 | 1285 | 0 |
| NSW | 18 | 0 | 149 | 0 |
| NT | 0 | 0 | 0 | 0 |
| QLD | 34 | 0 | 265 | 0 |
| SA | 2 | 0 | 19 | 0 |
| TAS | 0 | 0 | 210 | 0 |
| VIC | 1 | 0 | 268 | 0 |
| WA | 24 | 0 | 374 | 0 |

## Rabbit calicivirus

Rabbit calicivirus (RCV) has now been released at more than 780 sites around Australia (Table 7). The Rabbit Calicivirus Disease (RCD) Monitoring and Surveillance Program is responsible for assesssing the spread, persistence and impact of RCD. There are ten intensive sites where changes in rabbit populations, disease prevalence, flora, fauna, predators and agricultural production are measured. In addition, there are 54 broadscale sites where data on virus activity and rabbit abundance are collected.

As at February 1998, RCD has been recorded at all ten intensive monitoring sites. Rabbit numbers have remained low at seven out of nine of these sites where the initial RCD impact has been assessed. In the arid/semi-arid areas (less than 300 mm rainfall per year) 82% of the intensive and broadscale sites have recorded rabbit population declines of greater than 65%. By contrast, RCD appears to be less effective in reducing rabbit numbers in wetter areas where only 46% of the sites recorded similar declines.

*For further information contact Mr Mike Hillier, Executive Officer, RCD Program, Tel 02 6272 3425*

**Table 7: RCV releases to February 1998**

| | No. release sites | No. with obvious RCV activity | No. with no obvious RCV activity | No. not assessed |
|---|---|---|---|---|
| ACT | 8 | 2 | 3 | 3 |
| NSW | 485 | 269 | 132 | 84 |
| NT | 9 | 2 | 5 | 2 |
| Qld | 83 | 25 | 5 | 53 |
| SA* | 28 | 0 | 0 | 28 |
| Tas | 17 | 3 | 0 | 14 |
| Vic | 116 | 67 | 31 | 18 |
| WA | 41 | 13 | 20 | 8 |
| Total | 787 | 381 | 196 | 210 |

* Serological testing has shown that RCV had arrived at all sites in SA before deliberate release

## Laboratory testing

The results of serological testing from routine laboratory submissions for the quarter are shown in Table 8.

**Table 8: Serological testing from routine submissions to State laboratories**

| | Akabane | | Bluetongue | | Bovine ephemeral fever | | Enzootic bovine leucosis | | Equine infectious anaemia | | Equine viral arteritis | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tests | +ve | Tests | +ve | Tests | +ve | Tests | +ve | Tests | +ve | Tests | +ve |
| **Jan - Mar 97** | 729 | 197 | 5064 | 281 | 851 | 203 | 3256 | 147 | 416 | 0 | 287 | 4 |
| **Apr - Jun 97** | 796 | 144 | 6925 | 463 | 1426 | 528 | 5860 | 140 | 398 | 3 | 240 | 0 |
| **Jul - Sep 97** | 1011 | 285 | 7797 | 182 | 944 | 171 | 3231 | 28 | 348 | 0 | 279 | 1 |
| **Oct - Dec 97** | 2229 | 356 | 7442 | 332 | 1464 | 180 | 5228 | 2 | 710 | 1 | 462 | 26 |
| **Jan - Mar 98** | 1683 | 408 | 8005 | 224 | 2072 | 611 | 907 | 16 | 657 | 0 | 708 | 9 |
| **NSW** | 306 | 141 | 756 | 6 | 605 | 15 | 540 | 0 | 501 | 0 | 628 | 4 |
| **NT** | 397 | 115 | 702 | 111 | 458 | 109 | 0 | 0 | 0 | 0 | 0 | 0 |
| **QLD** | 415 | 120 | 4936 | 83 | 409 | 72 | 352 | 16 | 101 | 0 | 26 | 0 |
| **SA** | 2 | 0 | 301 | 0 | 2 | 0 | 0 | 0 | 8 | 0 | 2 | 0 |
| **TAS** | 2 | 0 | 6 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 0 |
| **VIC** | 126 | 0 | 164 | 0 | 147 | 0 | 0 | 0 | 0 | 0 | 17 | 5 |
| **WA** | 435 | 32 | 1140 | 24 | 451 | 415 | 7 | 0 | 47 | 0 | 35 | 0 |

# Surveillance activities

## Zoonoses

The National Notifiable Diseases Surveillance System of the Communicable Diseases Network Australia New Zealand collects statistics about many human diseases. The information is accessible at *http://www.health.gov.au/hfs/pubs/cdi/*. Table 9 summarises some of the information for zoonoses.

*Contributed by Communicable Diseases Intelligence, Department of Health and Family Services*

**Table 9: Notifications of zoonotic diseases in humans**

| Disease | Q1-97 | Q2-97 | Q3-97 | Q4-97 | Q1-98 | Current quarter | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Australia | | | | AUST | ACT | NSW | NT | QLD | SA | TAS | VIC | WA |
| **Brucellosis** | 12 | 4 | 13 | 13 | 13 | 0 | 1 | 0 | 11 | 0 | 0 | 1 | 0 |
| **Hydatidosis** | 6 | 13 | 22 | 19 | 9 | 0 | 0 | 0 | 2 | 3 | 0 | 4 | 0 |
| **Leptospiros** | 31 | 40 | 20 | 33 | 40 | 0 | 7 | 2 | 24 | 0 | 0 | 5 | 2 |
| **Listeriosis** | 23 | 22 | 14 | 9 | 18 | 0 | 16 | 0 | 1 | 1 | 0 | 0 | 0 |
| **Ornithosis** | 22 | 12 | 5 | 7 | 5 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 1 |
| **Q fever** | 139 | 166 | 136 | 124 | 114 | 0 | 50 | 0 | 53 | 1 | 0 | 2 | 8 |

## Northern Australia Quarantine Strategy

In recognition of the special quarantine risks associated with Australia's sparsely populated northern coastline, AQIS conducts an animal disease surveillance program as an integral component of the Northern Australia Quarantine Strategy (NAQS). The NAQS surveillance program provides early warning of disease threats to livestock industries, and in some cases human health. NAQS surveillance activities include both offshore and onshore components. Table 10 summaries NAQS activity over the past five quarters.

**Table 10: Summary of recent NAQS activity**

| | Q1-97 | | Q2-97 | | Q3-97 | | Q4-97 | | Q1-98 | | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Num | Pos | Num | Pos | Num | Pos | Num | Pos | Num | Pos | |
| Avian influenza | 0 | 0 | 48 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | a |
| Aujeszky's disease | 0 | 0 | 25 | 0 | 14 | 0 | 0 | 0 | 0 | 0 | |
| Classical swine fever | 0 | 0 | 50 | 0 | 64 | 0 | 50 | 0 | 9 | 0 | |
| Infectious bursal disease | 0 | 0 | 45 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | a |
| Japanese encephalitis | 319 | 3 | 320 | 2 | 128 | 0 | 209 | 0 | 450 | 27 | b |
| Newcastle disease | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Porcine reproductive and respiratory syndrome | 0 | 0 | 26 | 0 | 14 | 0 | 0 | 0 | 0 | 0 | |
| Screw-worm fly | 0 | 0 | 2 | 0 | 1 | 0 | 2 | 0 | 0 | 0 | |
| Swine influenza | 0 | 0 | 25 | 0 | 14 | 0 | 0 | 0 | 0 | 0 | |
| Surra | 83 | 0 | 105 | 0 | 102 | 0 | 136 | 0 | 34 | 0 | |
| Canine ehrlichiosis | 0 | 0 | 29 | 0 | 30 | 0 | 40 | 0 | 0 | 0 | |
| Transmissible gastroenteritis | 0 | 0 | 25 | 0 | 14 | 0 | 0 | 0 | 0 | 0 | |
| Trichinellosis | 5 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | |

**Notes**

**a** Serologically positive migratory birds that show clinical signs of disease are occasionally found during NAQS surveys (in this case, wandering whistle ducks trapped on Cape York Peninsula). Nevertheless, Australian domestic poultry flocks are free of these diseases.

**b** In previous years, sentinel sites on islands in the Torres Strait, but not on the Australian mainland, have shown seroconversions during the latter part of the wet season (January–April). In late March this year

seroconversions occurred at a number of sentinel sites on islands in the Torres Strait (Saibai, Badu, Moa and Mabuiag), and for the first time on the mainland, near Bamaga, at the tip of Cape York Peninsula.

There were two human cases of the disease in March this year — a 12-year-old unvaccinated child on Badu Island in the Torres Strait, and a professional fisherman, at the mouth of the Mitchell River on remote western Cape York Peninsula. Both patients have recovered.

Further pig testing and surveillance is being carried out. So far, seropositive pigs have been detected on Stephens, Darnley, Hammond and Prince of Wales Islands and in a single herd of domesticated wild pigs surveyed in the Mitchell River area. In all cases the pigs that seroconverted have shown no signs of disease. Further details will be provided in the next issue of this newsletter.

*Contact: David Banks, AQIS*

## Salmonella surveillance

The National Salmonella Surveillance Scheme (NSSS) is operated and maintained on behalf of the Commonwealth and States/Territories by the Microbiological Diagnostic Unit at the University of Melbourne. Data on isolates of salmonellae and other pathogens are submitted to the NSSS from participating laboratories around Australia.

Quarterly newsletters and annual reports of both human and non-human isolates are published, and detailed data searches are provided on request to the NSSS. Table 11 summarises *Salmonella* isolations from animals, notified to the NSSS for the quarter.

*Contributed by National Salmonella Surveillance Scheme, Microbiological Diagnostic Unit, University of Melbourne.*

**Table 11: Salmonella notifications, 1 January to 31 March 1998**

| Serovars | avian | bovine | canine | equine | feline | ovine | porcine | other | Total |
|---|---|---|---|---|---|---|---|---|---|
| *S. bovismorbificans* | 0 | 10 | 1 | 0 | 0 | 0 | 0 | 0 | 11 |
| *S. dublin* | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 24 |
| *S. infantis* | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| *S. typhimurium* | 12 | 42 | 3 | 3 | 0 | 2 | 1 | 5 | 68 |
| Other | 36 | 23 | 8 | 4 | 1 | 0 | 7 | 32 | 111 |
| Total | 48 | 99 | 13 | 7 | 1 | 2 | 8 | 37 | 215 |

## National Residue Survey

Table 12 summarises the results for the quarter. Information about the National Residue Survey (NRS) can be found on the worldwide web at *http://www.brs.gov.au/residues/residues.html* where there are sections on:

- NRS Business Plan 1997–98;
- NRS 1996 Randomised Sampling Results;
- recent publications;
- frequently asked questions;
- information for laboratories; and
- associated web sites.

Recent publications by the NRS include

- NRS Annual report 1996–97;
- Report on the 1996 NRS Results;
- Report on the 1995 NRS Results; and
- Report on the 1993–4 NRS Results.

Copies available from Dr Rusty Branford, NRS, PO Box E11, Kingston, ACT 2604

Phone 02 6272 5096
Fax 02 6272 4023
E-mail Rusty.Branford@brs.gov.au

*Contributed by National Residue Survey, Bureau of Resource Sciences*

**Table 12: National Residue Survey, 1 January to 31 March 1998**
Each pair of figures gives the number of samples above either the maximum residue limit or the maximum permitted concentration and the number of samples tested.

| | NSW | NT | QLD | SA | TAS | VIC | WA | AUST |
|---|---|---|---|---|---|---|---|---|
| **Antimicrobials** | | | | | | | | |
| cattle | 0 128 | 0 1 | 0 109 | 0 30 | 0 11 | 0 76 | 0 12 | 0 367 |
| pigs | 2 169 | 0 0 | 4 73 | 0 39 | 0 2 | 4 64 | 1 29 | 11 376 |
| poultry | 0 0 | 0 0 | 0 1 | 0 0 | 0 0 | 0 0 | 0 0 | 0 1 |
| sheep | 0 58 | 0 0 | 0 8 | 0 22 | 0 3 | 0 31 | 0 33 | 0 155 |
| other | 0 6 | 0 0 | 0 14 | 0 3 | 0 0 | 0 3 | 0 2 | 0 28 |
| Total | 2 361 | 0 1 | 4 205 | 0 94 | 0 16 | 4 174 | 1 76 | 11 927 |
| **Anthelmintics** | | | | | | | | |
| cattle | 0 89 | 0 0 | 0 104 | 0 14 | 0 12 | 0 73 | 0 13 | 0 305 |
| pigs | 0 34 | 0 0 | 0 14 | 0 3 | 0 1 | 0 14 | 0 6 | 0 72 |
| sheep | 0 147 | 0 0 | 0 12 | 1 62 | 0 8 | 0 105 | 0 66 | 1 400 |
| Total | 0 270 | 0 0 | 0 130 | 1 79 | 0 21 | 0 192 | 0 85 | 1 777 |
| **Growth promotants** | | | | | | | | |
| cattle | 0 167 | 0 1 | 0 147 | 0 24 | 0 18 | 0 114 | 0 46 | 0 517 |
| pigs | 0 11 | 0 0 | 0 8 | 0 4 | 0 0 | 0 3 | 0 2 | 0 28 |
| sheep | 0 95 | 0 0 | 0 14 | 0 56 | 0 12 | 0 80 | 0 72 | 0 329 |
| other | 0 1 | 0 1 | 0 12 | 0 3 | 0 0 | 0 3 | 0 0 | 0 20 |
| Total | 0 274 | 0 2 | 0 181 | 0 87 | 0 30 | 0 200 | 0 120 | 0 894 |
| **Insecticides** | | | | | | | | |
| cattle | 0 394 | 0 3 | 0 365 | 0 68 | 0 53 | 0 285 | 0 71 | 0 1239 |
| feral | 0 0 | 0 0 | 0 1 | 0 0 | 0 0 | 0 0 | 0 0 | 0 1 |
| pigs | 1 94 | 0 0 | 0 37 | 1 17 | 0 0 | 0 38 | 0 12 | 2 198 |
| poultry | 0 0 | 0 0 | 0 1 | 0 0 | 0 0 | 0 0 | 0 0 | 0 1 |
| sheep | 0 298 | 0 0 | 0 39 | 0 107 | 0 13 | 1 224 | 0 134 | 1 815 |
| other | 0 14 | 0 0 | 0 17 | 0 5 | 0 0 | 0 4 | 0 5 | 0 45 |
| Total | 1 800 | 0 3 | 0 460 | 1 197 | 0 66 | 1 551 | 0 222 | 3 2299 |
| **Metals** | | | | | | | | |
| cattle | 1 17 | 0 0 | 0 20 | 0 3 | 0 3 | 0 21 | 0 1 | 1 65 |
| pigs | 0 20 | 0 0 | 1 4 | 0 4 | 0 0 | 0 7 | 0 2 | 1 37 |
| sheep | 2 30 | 0 0 | 0 3 | 4 15 | 0 3 | 2 19 | 4 11 | 12 81 |
| other | 0 0 | 0 1 | 0 0 | 0 0 | 0 0 | 0 0 | 0 1 | 0 2 |
| Total | 3 67 | 0 1 | 1 27 | 4 22 | 0 6 | 2 47 | 4 15 | 14 185 |

## Suspect Exotic Disease Investigations

There were 23 exotic disease investigations reported during the quarter, as shown in Table 13.

**Table 13: Suspect exotic disease investigations**

| Disease | Species | State | Reponse (key below) | Finding |
|---|---|---|---|---|
| Bluetongue | ovine | NSW | 2 | Negative |
| Avian influenza | avian | NSW | 5 | Coccidiosis |
| Newcastle disease | avian | NSW | 0 | False alarm |
| Avian influenza | avian | NSW | 2 | Negative |
| Avian influenza | avian | NSW | 2 | Negative |
| Rabies | feline | NSW | 2 | Hepatic lipidosis and leucoencephalomalacia |
| Avian influenza | avian | NT | 2 | Fowl cholera |
| Bluetongue | ovine | QLD | 1 | Photosensitisation |
| Epizootic lymphangitis | equine | QLD | 2 | Investigation not yet complete |
| Avian influenza | avian | QLD | 3 | Marek's disease and mycoplasma |
| Bluetongue | ovine | QLD | 2 | Bluetongue excluded |
| New World screw-worm | canine | QLD | 2 | *Lucilia cuprina* |
| Bovine spongiform encephalopathy | bovine | QLD | 2 | Sporadic bovine encephalomyelitis |
| Horse pox | equine | QLD | 2 | Papilloma (Papova) virus |
| Avian influenza | avian | QLD | 3 | Coccidiosis |
| Bat lyssavirus | fauna | TAS | 3 | Negative |
| Avian influenza | avian | VIC | 1 | Chronic respiratory disease |
| Avian influenza | avian | VIC | 1 | Botulism |
| Bluetongue | ovine | WA | 2 | Negative |
| Vesicular stomatitis | bovine | WA | 2 | Negative |
| Newcastle disease | avian | WA | 2 | Negative |
| Duck virus enteritis | avian | WA | 2 | Negative |
| Bat lyssavirus | fauna | WA | 3 | Negative |

KEY:
Highest level of response:
1 Field investigation by Government Officer
2 Investigation by State or Territory Government veterinary laboratory
3 Specimens sent to the Australian Animal Health Laboratory (or CSIRO Division of Entomology)
4 Specimens sent to reference laboratories overseas
5 Regulatory action taken (quarantine or police)
6 Alert or standby

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